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VARIANTS IN USTILAGO NUDA AND CERTAIN HOST RELATIONSHIPS[1]

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INTRODUCTION

Previous to the investigations made by Tisdale and Tapke [2] it was thought that infection of barley by loose smut, *Ustilago nuda*, took place only through the young embryo at flowering time. These investigators found that certain varieties of barley became heavily infected by loose smut if dehulled seed was inoculated with spores of the fungus before sowing. Low percentages of infection were obtained when hulled seed was inoculated. The fact that seed-surface disinfectants control loose smut in some varieties, especially the six-rowed varieties, indicates that considerable infection may take place from seed-borne spores.[3] [4] Surface disinfectants, however, do not prove satisfactory in many cases, which indicates that considerable floral infection also takes place. In an unpublished report (1923) by R. S. Kirby, formerly extension pathologist of the New York Agricultural Experiment Station, it is stated that surface disinfectants, such as formaldehyde and the organic mercury compounds, control loose smut in six-rowed barleys but fail to control it in Alpha, a two-rowed barley grown extensively in New York. A news item furnished by the local farm bureau and published by the Sun of Norwich, N. Y., April 17, 1925, stated that surface disinfectants controlled loose smut in the six-rowed winter barleys but not in Alpha. The cause of these differences was not known. Climatic factors, morphological and physiological characters of the host, and variants or physiologic strains of the fungus were among the possible explanations. Investigations were started in the fall of 1924 in an attempt to answer some of the questions covering the action of loose smut on barley. The results of these investigations are reported herein.

METHODS AND MATERIALS

All seed for these experiments was obtained from smut-free plots of pure varieties of barley. Following methods previously used,[5] the

[1] Received for publication Nov. 18, 1926; issued July, 1927.

[2] TISDALE, W. H., and TAPKE, V. F. INFECTION OF BARLEY BY USTILAGO NUDA THROUGH SEED INOCULATION. Jour. Agr. Research (1924) 29: 263–284, illus. 1925.

[3] JOHNSON, A. G. EXPERIMENTS ON THE CONTROL OF CERTAIN BARLEY DISEASES. (Abstract) Phytopathology 4: 46. 1914.

[4] TISDALE, W. H., TAYLOR, J. W., and GRIFFITHS, M. A. EXPERIMENTS WITH HOT WATER, FORMALDEHYDE, COPPER CARBONATE, AND CHLOROPHOL FOR THE CONTROL OF BARLEY SMUTS. Phytopathology 13: 153–160. 1923.

[5] TISDALE, W. H. AN EFFECTIVE METHOD OF INOCULATING BARLEY WITH COVERED SMUT. Phytopathology 13: 551–554. 1923.

seeds were carefully dehulled before inoculation. The dehulled seeds were thoroughly shaken in envelopes or vials with sufficient spore material to render them dark in color. To prevent the mixing in the greenhouse of smut collections obtained from various sources, the smutted heads were inclosed in glassine bags. In collecting the smutted heads the stems were cut just below the bags. As soon as the smut was dry the spores were carefully rubbed from the heads inside without opening the bags. The outside of the bag was then thoroughly washed with a cloth wet with bichloride of mercury (1 : 1,000), and the spores placed in small bottles which were kept corked until it was time to inoculate the seed, which was done just before sowing. The seeds were carefully spaced 1½ or 2 inches apart in rows 6 inches apart on benches in a greenhouse at Arlington Experiment Farm, Rosslyn, Va. In some cases smut injured germination and weakened the seedlings to such an extent that it was necessary to break the surface of the soil and straighten the abnormal seedlings so that they could develop.

EFFECTS OF SMUT ON GERMINATION AND GROWTH

A description of the injury to germination and seedling development by *Ustilago nuda* has been given by Tisdale and Tapke.[6] This same type of injury was observed again in the present investigations. It was noted also that in 1924–25 fewer of the seedlings from inoculated seed grew to maturity than did those from uninoculated seed. Table 1 shows the numbers of seedlings from uninoculated and inoculated seed two weeks after sowing, the number of plants which matured from these seedlings, and the percentages of smutted plants for each variety tested.

Table 1 shows that the germination of the seed of the Nakano Wase variety was injured by the inoculation to a greater extent than that of any other variety, although with one exception no smut occurred in the mature plants. A high percentage of plants from inoculated seed of both Nakano Wase and Orel died before maturity, regardless of the appearance of any smut in the heads of plants which matured. Every variety tested showed some weakness as the result of seed inoculation.

Similar results were obtained in experiments in the winter of 1925–26, in which a larger number of smut collections were used. The effect of these various collections of smut on Tennessee Winter and Hannchen barleys, the only varieties on which all the smuts were used, is shown in Table 2. Tennessee Winter showed the most injury and Hannchen the least injury of all the varieties tested in this experiment.

[6] TISDALE, W. H., and TAPKE, V. F. INFECTION OF BARLEY BY USTILAGO NUDA THROUGH SEED INOCULATION. Jour. Agr. Research (1924) 29: 263–284, illus. 1925.

TABLE 1.—*Effect of inoculating dehulled seed of six different varieties of barley with spores of loose smut obtained from different sources*

[Test made in greenhouse at Arlington Experiment Farm, Rosslyn, Va. Seed planted November 20, 1924]

Variety and locality from which inoculum was obtained	Varieties inoculated, number of seedlings on Dec. 4, number of plants maturing, and percentage of smutted plants								
	Han River (C. I. 206)			Hannchen (C. I. 531)			Nakano Wase (C. I. 2166)		
	Number of seedlings	Number of mature plants	Percentage of smutted plants	Number of seedlings	Number of mature plants	Percentage of smutted plants	Number of seedlings	Number of mature plants	Percentage of smutted plants
	50 seeds			*50 seeds*			*50 seeds*		
Control [a]	48	43	0	46	39	0	42	39	0
Alaska (Rosslyn, Va.) [a]	45	37	16.2	33	28	0	20	13	7.7
Han River (Rosslyn, Va.) [a]	46	36	30.6	46	47	100.0	30	25	0
Unknown (Egypt) [b]	46	39	0	48	43	2.3	30	27	0
Manchuria (Madison, Wis.) [a]	44	28	7.1	40	33	0	24	20	0
Tennessee Winter (Rosslyn, Va.) [b]									

Variety and locality from which inoculum was obtained	Varieties inoculated, number of seedlings on Dec. 4, number of plants maturing, and percentage of smutted plants								
	Orel (C. I. 351)			Tennessee Winter (Selection 52)			C. I. 2222 (hulless)		
	Number of seedlings	Number of mature plants	Percentage of smutted plants	Number of seedlings	Number of mature plants	Percentage of smutted plants	Number of seedlings	Number of mature plants	Percentage of smutted plants
	50 seeds			*50 seeds*			*25 seeds*		
Control [a]	35	33	0	43	24	4.2	19	20	0
				100 seeds					
Alaska (Rosslyn, Va.) [a]	33	11	100.0	78	41	80.5	19	17	0
Han River (Rosslyn, Va.) [a]	35	21	85.7	80	51	82.4	20	15	6.7
Unknown (Egypt) [b]	38	21	0	84	58	8.6	19	19	0
Manchuria (Madison, Wis.) [a]	31	17	88.2	70	37	81.1	20	18	5.6
				50 seeds					
Tennessee Winter (Rosslyn, Va.) [b]				42	25	64.0			

[a] Smut obtained in 1924. [b] Smut obtained in 1923.

TABLE 2.—*Effect of inoculation with spores of loose smut on the germination of 40 dehulled seeds each of Tennessee Winter and Hannchen barleys, sown November 24, 1925, in the greenhouse at Arlington Farm, Rosslyn, Va.*

Smut lot No.	Number of seeds germinating on different dates and final percentages of smutted plants												
	Tennessee Winter (selection 52)						Hannchen (C. I. 531)						
	Dec. 1	Dec. 2	Dec. 3	Dec. 4	Jan. 6	Percentage of plants smutted	Nov. 30	Dec. 1	Dec. 2	Dec. 3	Dec. 4	Dec. 19	Percentage of plants smutted
Control	27	31	34	34	35	0	22	37	39	39	39	40	0
1	16	26	27	28	36	0	18	30	34	34	33	40	0
2	9	10	16	17	32	85.7	12	33	38	38	38	40	0
3	8	21	22	28	34	72.7	15	35	39	39	40	40	72.5
4	20	28	32	33	37	0	19	30	32	34	34	37	0
5	4	10	11	13	29	78.6	14	30	37	37	37	38	97.4
6	2	11	12	10	22	68.2	12	26	31	34	32	34	97.0
7	7	13	17	17	34	93.6	4	17	27	29	30	39	0
8	4	14	16	18	35	71.4	8	31	39	40	40	40	100.0
9	11	24	28	29	36	81.8	8	30	36	37	37	39	0
10	5	9	14	14	33	75.8	5	31	38	40	40	40	95.0
11	2	10	14	15	32	75.9	13	31	36	37	37	36	91.7
12	19	28	31	32	35	0	23	37	37	37	37	40	0
13	21	29	30	30	36	0	27	38	39	39	39	39	0
14	7	15	16	16	26	84.0	18	36	37	39	38	40	0
15	21	28	29	30	34	0	28	38	40	40	40	40	0
16	9	19	21	22	35	77.8	26	39	40	40	40	40	0
Control	25	32	34	35	40	0	34	40	40	40	40	40	0
17	8	18	23	23	34	66.7	15	35	37	37	37	39	92.3
18	15	28	32	33	35	0	27	35	37	37	37	37	0
19	5	15	22	22	28	96.0	16	35	37	37	37	39	0
20	6	19	23	23	32	96.8	19	36	37	38	38	40	0
21	5	21	25	27	32	93.6	16	37	38	38	38	38	0
22	12	20	25	25	33	90.9	28	36	37	38	38	40	0
23	7	15	18	19	33	72.4	18	35	37	38	38	39	0
24	6	15	17	16	29	74.1	23	31	33	33	34	38	0
25	6	15	19	20	32	81.3	23	35	36	37	38	39	0
26	6	16	19	20	30	86.2	29	36	36	37	37	38	0
27	5	15	20	19	34	90.3	28	35	37	38	38	40	0
28	8	19	24	24	32	92.9	29	35	37	37	37	* 39	2.6
29	12	19	26	28	31	93.1	31	38	38	38	38	* 40	0
30	6	14	19	21	34	90.6	30	37	37	37	37	* 40	0
31	5	17	22	23	30	85.7	31	36	37	37	37	* 39	0
32	19	31	31	31	35	2.9	30	32	32	33	33	* 35	5.7
Control	31	33	34	35	37	0	39	39	40	40	40	* 40	0

* Record made Dec. 18.

In smut lots Nos. 1, 4, 12, 13, and 15 germination of Tennessee Winter was retarded less than the others. Even these lots, however, showed some retardation as compared with the controls. The plants from the control rows were smut free. In all other smut lots germination and development of the plants were affected. Hannchen was injured less than Tennessee Winter. Some of the most severe injury to germination occurred in smut lots which produced no smutted heads in the mature plants. Smut lot No. 7 is an example. This collection showed a marked effect on germination and the early stages of seedling development in Hannchen, but heads of the mature plants were smut free. The same smut, however, produced over 93 per cent of smutted plants in Tennessee Winter. Plants from inoculated seed which failed to become smutted were cut back near the surface of the soil after the smut records were made. A few of them produced secondary culms. Three plants of Han River and one of Tennessee Winter produced smutted heads in the secondary growth. In view of these results it is obvious that the term "infection" can not be used accurately to include only the

appearance of smutted heads in mature plants. Plants may be heavily infected and show no smut at maturity. The effect of the fungus on germination and plant development may prove of value also in studying strain differences in loose smut of barley.

SMUT STRAINS AS INDICATED BY PERCENTAGES OF SMUTTED PLANTS

In addition to the information on germination and plant development, Table 1 gives the percentages of smutted plants. One item of interest shown in this table is the occurrence of 100 per cent of loose smut in Hannchen grown from seed inoculated with smut spores from Han River, while plants of the same variety grown from seed inoculated with spores from Alaska were smut free. Of further interest, however, is the fact that Alaska smut spores produced high percentages of the disease in other varieties. Both the Alaska and Han River plants, from which the inoculum for Hannchen was collected, were grown in the greenhouse from dehulled seed inoculated with spores taken from the same collection of Tennessee Winter smut in 1923. There seemed to be two possible explanations for the occurrence or absence of smut in Hannchen, either one or both of which may be concerned. The first and apparently the more probable explanation is that two strains of smut occurred in the original collection from Tennessee Winter barley, one producing smut in Alaska but not in Hannchen and the other producing smut in both Hannchen and Han River but not in Alaska. The second is that there was only one strain of the smut in the original collection and that the fungus was influenced by Alaska in some way which prevented it from producing smut in Hannchen. Further data bearing on these points were obtained in a later experiment. The smut from Alaska produced 100 per cent of smut in Orel. Likewise the spores collected from Manchuria barley at Madison, Wis., in 1924 (Table 1) which failed to produce smut in Hannchen produced over 88 per cent of smutted plants in Orel. Tennessee Winter was the only variety in which all collections of spores produced smut.

In the more comprehensive experiment in 1925–26 thirty-two collections of smut were used to inoculate several varieties of barley. Table 3 gives the source and varieties of barley in which each collection of smut was grown since the original collection was made, as well as the smut percentages for Tennessee Winter and Hannchen and in some cases for other varieties inoculated in these experiments.

In no case as shown in Table 3, did a collection of smut which failed to produce smutted plants in Tennessee Winter produce the disease in any other variety of barley inoculated. Spores from many of the collections which produced high percentages of smut in Tennessee Winter failed to produce smutted heads in Hannchen. None of the three smuts used to inoculate Nakano Wase produced smutted plants, but the three smuts used on Alpha, the six smuts used on Han River, and the seven smuts used on Alaska all produced high percentages of smutted heads. Two of the three smuts used to inoculate Orel were effective.

As shown in Table 1, spores from Han River barley which had been inoculated with smut from Tennessee Winter barley produced 100 per cent of smut in Hannchen, whereas spores from Alaska barley

TABLE 3.—*Effect of inoculating dehulled seed of seven varieties of barley with spores of loose smut obtained from different sources.* [*Test made in greenhouse at Arlington Farm, Rosslyn, Va., winter of 1925–26*]

Smut lot No.	Source of smut	Varieties from which smut was obtained in—			Percentage of smutted plants in different varieties						
		1923	1924	1925	Tennessee, Winter (selection 52)	Hannchen (C. I. 531)	Alaska (C.I.4106)	Han River (C. I. 206)	Orel (C. I. 351)	Alpha (C. I. 959)	Nakano Wase C.I.2166)
Control					0	0	0	0	0	0	0
1	Rosslyn, Va	Tennessee Winter			0	0					
2	do		Alaska	Tennessee Winter	85.7	0					
3	Moorhead, Minn			Oderbucker	72.7	72.5					
4	Rosslyn, Va			Nakano Wase	0	0					0
5	do	Tennessee Winter	Han River	Han River	78.6	97.4	42.1	46.2		92.1	
6	do	do	do	Hannchen	68.2	97.0	60.0	51.3			
7	do	do	Alaska	Orel	93.6	0	59.0	77.1	58.3		
8	do	do	Han River	do	71.4	100.0	57.5	35.9	67.5		
9	Madison, Wis		Manchuria	Tennessee Winter	81.8	0					
10	Rosslyn, Va	Tennessee Winter	Han River	do	75.8	95.0					
11	do	do	do	Hannchen	75.9	91.7					
12	Madison, Wis		Wisconsin P. 9	Wisconsin P. 9	0	0					
13	do		Manchuria	Orel	0	0			0		
14	Rosslyn, Va	Tennessee Winter	Han River	Alpha	84.0	0				79.0	0
15	Ames, Iowa			Hooded	0	0					
16	Corvallis, Oreg			Alaska	77.8	0	71.1			84.6	0
Control					0	0	0	0	0	0	0
17	Madison, Wis		Manchuria	Tennessee Winter	66.7	92.3					
18	do		Wisconsin P. 6	Wisconsin P. 6	0	0					
19	Rosslyn, Va	Tennessee Winter	Han River	Tennessee Winter	96.0	0					
20	Egypt		Unknown	do	96.8	0					
21	Rosslyn, Va	Tennessee Winter	Alaska	do	93.6	0					
22	do	do	do	do	90.9	0					
23	do	do	do	do	72.4	0					
24	do	do	do	do	74.1	0					
25	do	do	do	do	81.3	0					
26	do	do	Han River	do	86.2	0					
27	do	do	do	do	90.3	0					
28	do	do	do	do	92.9	2.6					
29	do	do	do	do	93.1	0					
30	do	do	Alaska		90.6	0	62.5	69.2			
31	do	do	Han River		85.7	0	55.0	79.5			
32	Madison, Wis		Manchuria		2.9	5.7					
Control					0	0					

which had been inoculated with the same collection from Tennessee Winter failed to produce smutted plants in Hannchen. The possible occurrence of two strains of smut in the original collection and the influence of the host on the parasite were mentioned as possible explanations of the results of the inoculations. Table 3 shows that seed of Alaska inoculated with smut from Hannchen which came from the Han River variety, the latter in turn having been inoculated from the original Tennessee Winter collection, produced plants which were 60 per cent smutted, whereas in no case did seed of Hannchen inoculated with spores from Alaska or with spores from a smut collection that had been grown in Alaska and then in another variety produce smutted plants. The fact that smut from Hannchen produced smut in Alaska, while the smut in Alaska which came from the same original source as that in Hannchen failed to produce smut in Hannchen, tends to weaken the theory of distinct strains of the fungus specific to Alaska and Hannchen, which the data in Table 1 seem to support. It does seem to strengthen the theory that there is a definite host influence on the fungus which is shown when the smut is transmitted from one variety to another. As previously mentioned, both factors may be concerned in the results. Further study is needed to clear up these points.

A preliminary experiment in 1925 showed that Alpha barley, although apparently infected naturally by loose smut only through the flower, can be infected by inoculating the dehulled seed with loose-smut spores. Spores collected from Alpha in New York in 1924 failed, however, to produce smut either in Alpha or Han River when the dehulled seed was inoculated. The spores might not have been sufficiently viable to produce infection, or it may be possible that a strain of loose smut exists in New York which for some reason infects only through the young embryo at flowering time. On the other hand, there may be morphological factors of the host which prohibit infection in nature except through the flower, even though the dehulling of the seed would render the seedling very susceptible to infection by seed-borne spores. The three smuts used to inoculate Alpha in the experiment covered by Table 3 produced high percentages of smut in that variety.

Several barley varieties were inoculated from the original collection of loose smut on Manchuria from Madison, Wis., in 1924. In the fall of 1925 two collections of this same smut from Tennessee Winter and one from Orel were used in making the inoculations. One collection from Tennessee Winter produced smut in both Tennessee Winter and Hannchen, although Hannchen inoculated with spores from the original collection failed to become smutted. The other collection produced smut in Tennessee Winter but not in Hannchen. The collection from Orel failed to produce smut in either Tennessee Winter or Hannchen. This may be due to the occurrence of more than one strain of smut in Manchuria or to some other cause. An occasional mutation would explain occurrences of this kind.

Smut from the 1923 collection from Tennessee Winter, which subsequently had been grown in Han River and Alpha, failed to produce smut in Hannchen, but produced high percentages of the disease in Alpha and Tennessee Winter. Perhaps Alpha is smutted by the strain which is effective on Alaska but not on Hannchen or a similar

strain, or it alters the fungus in the same way that Alaska does so that it does not produce smut in Hannchen.

Only one plant of Nakano Wase was smutted in the experiments recorded in Table 1. Previous experiments had given negative results. In 1925, however, a number of heads of loose smut were found in Nakano Wase barley on Arlington Farm. Spores from these smutted heads were used in the experiments recorded in Table 3. No smut was produced in any variety by inoculating the seed. Although the spores were collected and handled with the same care as those of other collections, they might have lost sufficient of their vitality to prevent their causing infection at the time the seed was inoculated and sown. In an experiment conducted by J. W. Taylor and the writers, several heads of Nakano Wase were inoculated by placing the spores in the flower at the time the smutted heads were collected. The seed from the inoculated heads was sown on Arlington Farm the following season. From this seed 26 plants matured, of which 8, or 30.8 per cent, were smutted. As previously mentioned, the spores of some smut strains may remain viable longer than spores of other strains. There may also be strains which infect only through the flower.

CONCLUSIONS

The data obtained in these experiments show that variants exist in the species, *Ustilago nuda*. These variants apparently correspond to what in other fungi are termed strains or physiologic forms. There is some indication, however, that these variants are altered in their pathogenicity by certain hosts. Further study is needed to clear up this point. Various smut collections not only showed differences in their ability to produce smutted heads in barley varieties, but they also affected germination and host development differently. In studying the loose smut of barley it is important that its effects on the host plant throughout its entire development be studied. There may be strains of the loose smut which infect barley in nature only through the floral organs. This might be due either to morphological or physiological characters of the host, to short-lived spores, or to other physiological characters of the fungus. Further experimental study of the subject is needed.

DEVELOPMENT OF THE FEMALE GAMETOPHYTE AND CARYOPSIS OF POA PRATENSIS AND POA COMPRESSA[1]

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INTRODUCTION

As a result of the work of Cieslar, 1883 (*3*),[2] Von Liebenberg, 1884 (*18*), Jönsson, 1893 (*14, p. 40–47*), Hite, 1919 (*11*), Harrington, 1923 (*10*), Toole, 1923 (*29*), and many others, it is known that the seeds of *Poa pratensis* (Kentucky blue grass) require an alternation of temperatures for germination. An exposure of the seeds[3] on moistened blotters at a temperature of about 30° C. for 6 hours, and of 20° for 18 hours each day, results in complete germination of the viable seeds in about 28 days. An exposure of the seeds of *Poa compressa* (Canada blue grass) to a similar daily alternation of temperatures, and to light, and treatment with a dilute nitrate solution, result in complete germination of the viable seeds within three or four weeks.

This investigation was conducted in an effort to discover some of the causes underlying the differences in the germination conditions of the seeds of these two related species. A study of the development of the ovule and especially of the embryo was undertaken to determine whether the structure and stage of development of the embryo have any relation to germination conditions. Since polyembryony is frequently observed in seeds of Poa, it was thought desirable to trace the origin of the embryos where more than one is present. A microchemical study of the membranes of the caryopsis showed that during its development many of its structures are so modified that they can no longer be definitely identified in the mature caryopsis. It was necessary, therefore, to make a detailed comparative study of the development of the fruits of these two species, as well as a microchemical study of the membranes of the caryopses, preliminary to a more detailed investigation of the physiology of the germination of these seeds.

No noteworthy differences were found in the development and structure of *Poa pratensis* and *P. compressa;* hence the two species will be described together.

MATERIALS AND METHODS

During the summer periods of 1922, 1923, and 1924 material from plants of *Poa pratensis* and *P. compressa* growing in the vicinity of Washington, D. C., was collected at different stages of development of the panicles.

In January, 1923, and January, 1924, plants of both species were potted and placed in artificially lighted greenhouses at a temperature of 50 to 60° C. Artificial light was used to increase the light period.

[1] Received for publication Aug. 4, 1926; issued, July, 1927.

[2] Reference is made by number (italic) to "Literature cited," p. 1017.

[3] The word "seed" is used here to mean the caryopsis inclosed in the lemma and palea.

The panicles of *Poa pratensis* were in open flower about 2 months after potting. *Poa compressa* flowered 10 days later than *P. pratensis*. Material was collected at frequent intervals before the flowers opened. Each panicle was tagged the day it showed open flowers and a branch or two from a panicle was fixed twice daily at first, and less frequently as the seeds approached maturity. The material was fixed in chromo-acetic, picro-acetic, picro-chromic, Bouin's modified, Carnoy's, acetic alcohol, and Flemming's (weak, medium, and strong) solutions. Xylol was used to precede the paraffin infiltration. Sections were cut 8 to 12 microns thick. Flemming's triple stain, Ehrlich's haematoxylin and safranin, and Haidenhain's haematoxylin were used to stain the slides.

DEVELOPMENT OF THE FEMALE GAMETOPHYTE

Poa pratensis and *Poa compressa* have a simple ovary containing a single ovule. The ovary has two styles. As in other Gramineae the ovule is campylotropous (pl. 1, A). It has two integuments, each of which is composed of two layers of cells except at the base, where there are sometimes three layers (pl. 1, A).

The megaspore mother cell divides and forms two daughter cells which divide again and give rise to a row of four megaspores. Usually the innermost megaspore develops and forms the embryo sac, the other three being digested and absorbed by its growth (pl. 2, A). In several ovules observed, the outermost megaspore develops and forms the embryo sac. Frequently two embryo sacs develop within the same nucellus (pl. 2, C). Details of their development are discussed later. The female gametophyte develops in the usual manner. A two-nucleate gametophyte is shown in Plate 2, B. In the mature gametophyte, the two synergids and the egg nucleus are at the micropylar end, the two polar nuclei lie side by side at the center, and the three very large antipodals lie near the chalaza (pl. 1, B). The egg cell is sometimes spherical and sometimes pear-shaped.

Fertilization was not observed by the writer, although the slides were carefully examined for this detail. The material is not particularly favorable for the study of fertilization, because of its small size. The pollen mother cells have been observed to divide in the usual manner, forming the four microspores which appear perfectly normal. With reference to *Poa pratensis* Nishimura (*23*) says that the pollen grains show no departure and develop and germinate normally.

EXPLANATORY LEGEND FOR PLATE 1

A.—*Poa pratensis*. Longitudinal section of an immature ovary, showing 4 megaspores surrounded by nucellus; inner integument composed of 2 layers of cells; outer integument composed of 2 layers of cells; ovary wall. × 210

B.—*Poa compressa*. Longitudinal section of the female gametophyte surrounded by the nucellus, showing the egg cell and 2 synergids at the micropylar end; 2 polar nuclei at the center; 3 comparatively small antipodals near the chalaza. × 500

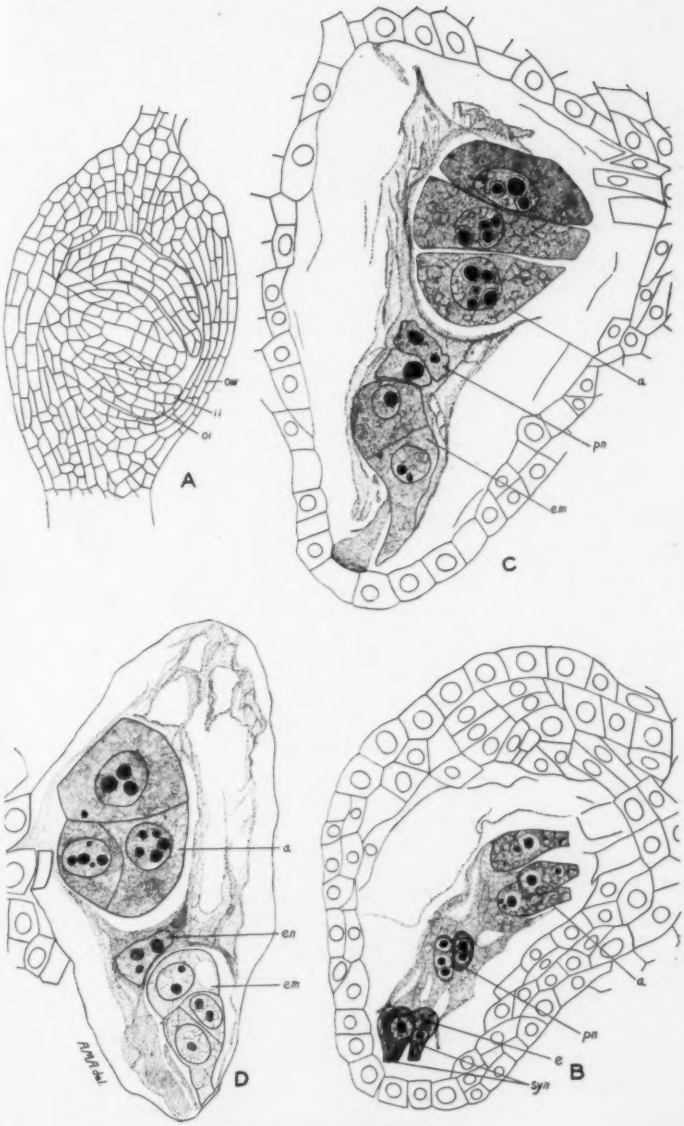
C.—*Poa compressa*. Longitudinal section of the embryo sac showing a two-celled embryo at the micropylar end; 2 polar nuclei at the center; 3 very large antipodals near the chalaza. × 500

D.—*Poa compressa*. Longitudinal section of the embryo sac, showing a three-celled embryo at the micropylar end; 2 polar nuclei fused to form the endosperm nucleus at the center; 3 large antipodals near the chalaza. × 500

KEY TO ABBREVIATIONS USED IN PLATES AND FIGURES

a, antipodals; *pn*, polar nuclei; *en*, primary endosperm nucleus; *e*, egg; *syn*, synergids; *em*, embryo (when more than one embryo sac is present the parts of the embryo sac more distant from the micropyle are designated by *a'*, *pn'*, *en'*, *e'*, *syn'*, *em'*); *sc*, scutellum; *vs*, vascular strand; *cl*, coleoptile; *fl*, foliage leaves; *v*, vegetative cone; *ep*, epiblast; *r*, radicle; *rc*, root cap; *co*, coleorhiza; *ii*, inner integument; *oi*, outer integument; *nu*, nucellus; *ch l*, chlorophyll layer; *ow*, ovary wall; *s*, suberin; *c*, cutin; *ec*, epidermal cells.

In some of the photomicrographs the cell walls and the outlines of the nuclei have been traced in ink.



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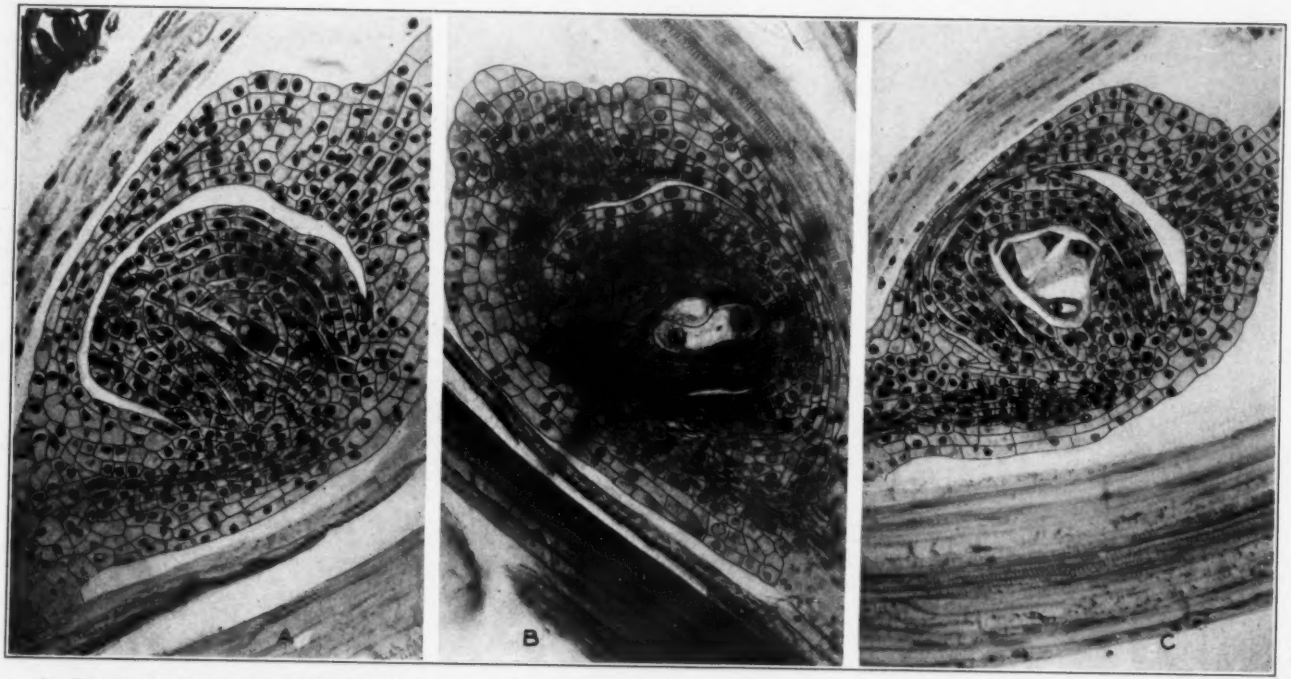


A.—*Poa pratensis*. Photomicrograph of a longitudinal section of a young ovary, showing four megaspores. The deepest megaspore is developing to form the embryo sac. ×250

B.—*Poa pratensis*. Photomicrograph of a longitudinal section of a young ovary, showing two embryo sacs lying side by side within the same nucellus. One embryo sac is two-nucleate, the other is one-nucleate, but its nucleus is not visible in this section. ×200

C.—*Poa pratensis*. Photomicrograph of a longitudinal section of a young ovary, showing two embryo sacs within the same nucellus. Each embryo sac is in the two-nucleate stage. ×200

ANTIPODALS

The marked development of the antipodal cells is a notable feature in *Poa compressa* and *P. pratensis;* in fact, the Gramineae are conspicuous for their strongly developed antipodals. Johannsen, working on Hordeum (*15*), Koernicke on Triticum (*17*), and Cannon on *Avena fatua* (*1*), found that the antipodals increase in number to 36 or more before fertilization and begin to disorganize with the beginning of the endosperm development.

With the growth of the embryo sac, the antipodals of *Poa compressa* and *P. pratensis* increase greatly in size in comparison to the egg apparatus. Their position at the chalaza, which is supplied with nutriment through the vascular strand, insures a plentiful supply of food for their growth (pl. 1, C). Each antipodal contains a very large nucleus with several nucleoli. The antipodals are densely filled with protoplasm and take a very deep stain. They contain a few vacuoles even in the earlier stages (pl. 3, A, pl. 4. B), but more as they disintegrate. Three very large antipodals are generally associated with one embryo (pl. 1, B, C, D). However, four and five antipodals are found very frequently (pl. 3, A, and pl. 5, A). Although the antipodals of Poa do not increase in number to the same extent as the antipodals described by Johannsen in Hordeum, Koernicke in Triticum, and Cannon in *Avena fatua*, they are perhaps more conspicuous by their great size and dense protoplasm.

When two embryo sacs are found in the same nucellus, six or more antipodals are always present. In Plate 3, B, the three very large antipodals are associated with the embryo at the micropyle, and the other five antipodals overlying these three are associated with the second embryo more distant from the micropyle. Although the seeds showing polyembryony might at first glance be interpreted as containing a great increase in number of antipodals, close observation shows that they conform to two or more sets of three or more antipodals for each embryo sac (pl. 4, B). In one case (pl. 6, C), the antipodals (associated with the micropylar embryo and the endosperm filling one-half of the seed) are practically disintegrated, while the four antipodals (associated with an embryo not visible in the photomicrograph, and an undivided endosperm nucleus) persist very prominently.

The antipodals persist until late in the endosperm formation, when they become vacuolated and disintegrate. They are finally crowded off to one side and are presumably digested and absorbed by the growing endosperm.

DEVELOPMENT OF THE ENDOSPERM

By a series of rapid divisions the primary endosperm nucleus forms many free nuclei in a parietal layer of cytoplasm and in a mass about the embryo (pl. 6, A). A parietal layer of cells soon forms. The cells of the parietal layer divide, forming quite definitely two rows of cells except toward the embryo, where the cells divide more rapidly. Each cell of these two rows then divides again, filling in toward the central cavity. Gordon (*8*) found that in the formation of the endosperm of wheat, barley, and oats "the lining layer of the embryo sac assumes the character of a cambium, which

produces segment cells only on its inner surface." In *Poa pratensis* and *P. compressa* the cells of the inner row are equally as active in division as the cells of the outer row. Practically every cell of the inner row has been observed in a stage of division.

When endosperm formation is practically complete, the cells of the outermost layer of the endosperm divide, and the outer row of cells arising from this division differentiates to form the aleurone layer. The aleurone layer is composed of only one layer of cells, but occasionally two cells are superimposed one above the other. The aleurone cells surround the endosperm and embryo except at the base of the embryo.

There are several rows of nucellar cells present which are about the same size as the aleurone cells when the aleurone cells are first differentiated. The outermost nucellar cells are the only ones that form a definite and distinct row. At maturity two or more rows of nucellar cells remain and are compressed like the cells of the inner integument.

DEVELOPMENT OF MORE THAN ONE EMBRYO SAC WITHIN THE SAME NUCELLUS

Polyembryony occurs frequently in *Poa pratensis* and *P. compressa*. This was also observed by Zinn (*33*) in the germination of both of these species and by Nishimura (*23*) in the germination of *P. pratensis*. A review of the literature on polyembryony is given by Ernst (*6*). Polyembryony has been described as arising in various ways by many authors.

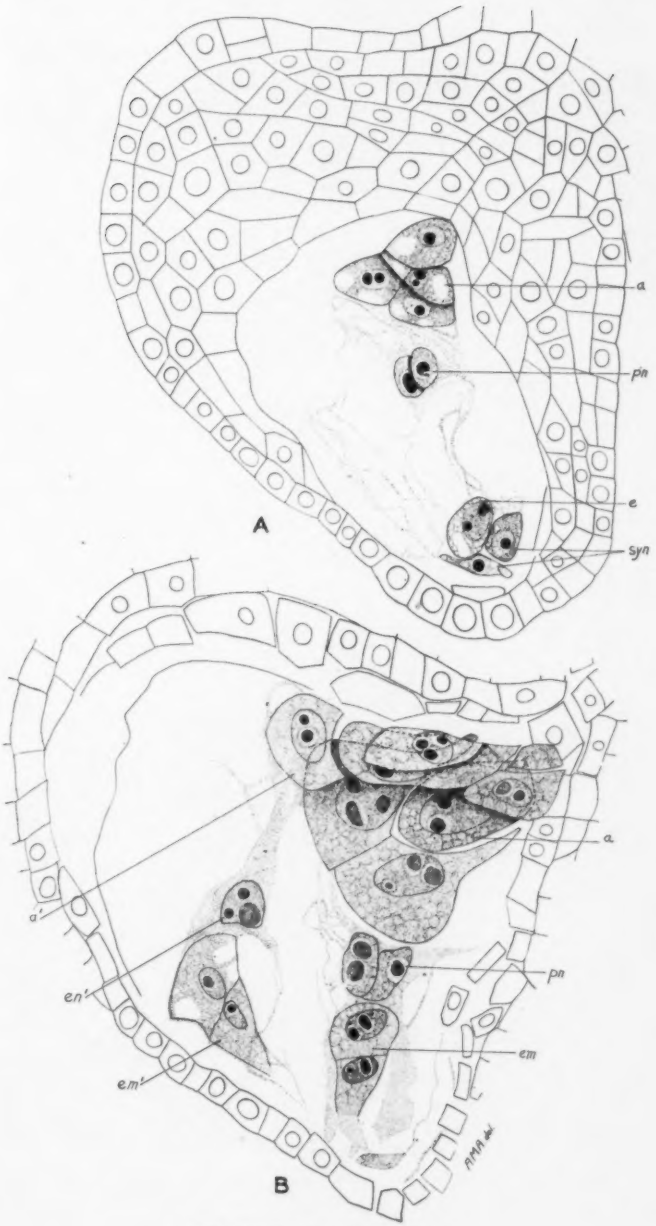
There is much well-illustrated literature on polyembryony in which embryos are described as arising from the nucellus, suspensor, and synergids. The presence of more than one embryo sac within the same nucellus is briefly mentioned by various authors, but except in rare instances only one embryo sac is thought to develop an embryo. In *Rosa lívida*, Strasburger (*28*) noted more than one embryo sac within the ovule, but found that only one developed. Schwere (*26*) states that in *Taraxacum officinale* in one case he found within one ovary two well-developed embryo sacs, each containing a normal healthy embryo with the cotyledons differentiated. Schacht (*25*) found that in *Cherianthus cheiri* several embryo sacs are developed, only one of which, the largest, becomes fertilized. Hofmeister (*12*) observed in Rosa sp. three to six embryo sacs only two of which usually contained egg cells. Jönsson (*13*) states that in *Trifolium pratense* more than one megaspore develops, and that more than one embryo sac within the same nucellus contains an egg which is fertilized. Mottier (*22*) observed in Delphinium two mature embryo sacs lying side by side in one ovule, but did not determine whether more than one embryo

EXPLANATORY LEGEND FOR PLATE 3

A.—*Poa pratensis*. A composite drawing of successive longitudinal sections of the embryo sac surrounded by the nucellus, showing egg cell and 2 synergids at the micropylar end; 2 polar nuclei at the center; 4 antipodals near the chalaza. × 500

B.—*Poa pratensis*. A composite drawing from successive longitudinal sections of a young ovary, showing 2 embryo sacs within the same nucellus. One embryo sac has developed a two-celled embryo at the micropyle, 2 polar nuclei at the center, and 3 very large antipodals near the chalaza. The other embryo sac also has developed a two-celled embryo slightly farther from the micropyle, endosperm nucelus at the center, and 5 long antipodals near the chalaza, somewhat overlying the antipodals of the other embryo sac. × 500

For key to abbreviations, see page 1002.



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was fertilized and whether more than one embryo reached maturity in a single seed. In the genus Smilacina McAllister (*19*) occasionally found within the same nucellus two embryo sacs which arose from two megaspore mother cells. These megaspore mother cells are usually separated more or less by somatic cells. Martin (*20*) found that in *Medicago sativa* the number of archesporial cells ranged from one to six and that more than one usually occurs. Two to four rows of megaspores may occur in the same nucellus and often more than one megaspore starts to form an embryo sac, but not more than one embryo sac matures.

The results of the present study seem to indicate that polyembryony in *Poa pratensis* and *P. compressa* results from the development of more than one embryo sac within the same nucellus. With the development of the megaspores, the young embryo sacs are crowded from their original positions in such a manner that it is difficult to determine whether they originate from two separate megaspore mother cells or from two megaspores of a single row of megaspores (pl. 2, C). In one ovule two parallel rows of four megaspores each were observed. In another ovule two 1-nucleate embryo sacs were found lying side by side. In another ovule (pl. 2, B) a 1-nucleate and a 2-nucleate embryo sac were present side by side. However, in two other ovules two 1-nucleate embryo sacs were observed one above the other, apparently in the same row. The two megaspores develop in the usual manner. Each of the well-developed embryo sacs contains an egg cell and two synergids at the micropylar end, the two polar nuclei at the center of the embryo sac, and three or more large antipodals near the chalaza. With the growth of the ovule the two embryo sacs usually lie side by side diagonally across the ovule from micropyle to chalaza. In a later stage of the development of the embryo sac, the one embryo often lies at the micropyle and the other embryo slightly above it, as shown in Plate 3, B, and Plate 4, A. The position of the embryo sacs relative to the chalaza seems to be an important factor in their development. If the embryo sacs are situated equally near to the chalaza and vascular strand, they develop simultaneously. The embryo sac (usually the one at the micropyle), which is situated nearer to the source of food, develops more rapidly. This condition is shown in Plate 7, A, where the embryo sac farther from the micropyle, which is in the 8-nucleate stage, is pushed up above the chalaza and the vascular strand, while the embryo sac at the micropyle is situated nearer the vascular strand and has developed an embryo of three or more cells. In many of the seeds both embryos are at about the same stage of development and each set of antipodals is in close contact with the chalaza (pl. 3, B, and pl. 4, A).

EXPLANATORY LEGEND FOR PLATE 4

A.—*Poa pratensis*. Photomicrograph of a longitudinal section of an immature ovary, showing 2 embryo sacs within the same nucellus. One embryo sac contains a two-celled embryo at the micropyle, 2 polar nuclei, and only 2 antipodals visible in this section near the chalaza. The other embryo sac contains an embryo of 9 or more cells slightly above the micropyle, endosperm nucleus, and 3 antipodals near the chalaza. × 220

B.—*Poa pratensis*. Photomicrograph of a longitudinal section of a young ovary, showing 2 embryo sacs within the same nucellus. One embryo sac contains an embryo at the micropyle but not visible in this section, 2 polar nuclei, and 4 antipodals near the chalaza. The other embryo sac contains an embryo of 8 or more cells slightly above the micropyle, endosperm nucleus, and 3 antipodals near the chalaza. × 220

In the early stages a developing endosperm is associated with each embryo, although both endosperms may not continue to develop (pl. 6, B). At this stage of development, as shown in Plate 6, B, one seed was found containing three embryos each associated with a peripheral layer of endosperm. At a slightly later stage in the development of the embryos several seeds showed the two embryos about equally developed, the endosperm of the embryo at the micropyle filling one-half of the nucellar cavity, while the endosperm nucleus of the embryo more distant from the micropyle had not divided (pl. 7, B, and pl. 6, C). In these seeds the endosperm nucleus of the embryo sac farther from the micropyle does not appear to have much chance of developing further, as the antipodals (from which the endosperm is probably supplied with food) are pushed away from the vascular strand. A later stage was observed in which the endosperm associated with the embryo at the micropyle filled three-fourths of the nucellar cavity, and the endosperm nucleus associated with the embryo farther from the micropyle was pushed to the distal end of the seed, away from the embryo that was previously connected with it.

In the sections of all the mature seeds in which more than one embryo was observed, each embryo was found to be complete in itself (pl. 8, C). The two mature embryos are in the same relative position as the younger embryos, both near the micropyle, one slightly above the other. Only one endosperm surrounded by the aleurone layer is generally observed in the mature seed. Some seeds containing two embryos were observed, but with one endosperm and the aleurone layer almost filling the nucellar cavity. However, a smaller endosperm was present which in one instance occupied a small space toward one side and in other instances was almost completely surrounded by the larger endosperm.

Seeds were observed in which in one case a two-nucleate, in another case a four-nucleate, and in still another case an eight-nucleate embryo sac was surrounded by the antipodals of a further developed embryo sac. A four-nucleate embryo sac surrounded by the antipodals associated with the embryo at the micropyle, is shown in Plate 5, B. Such embryo sacs probably do not develop two embryos.

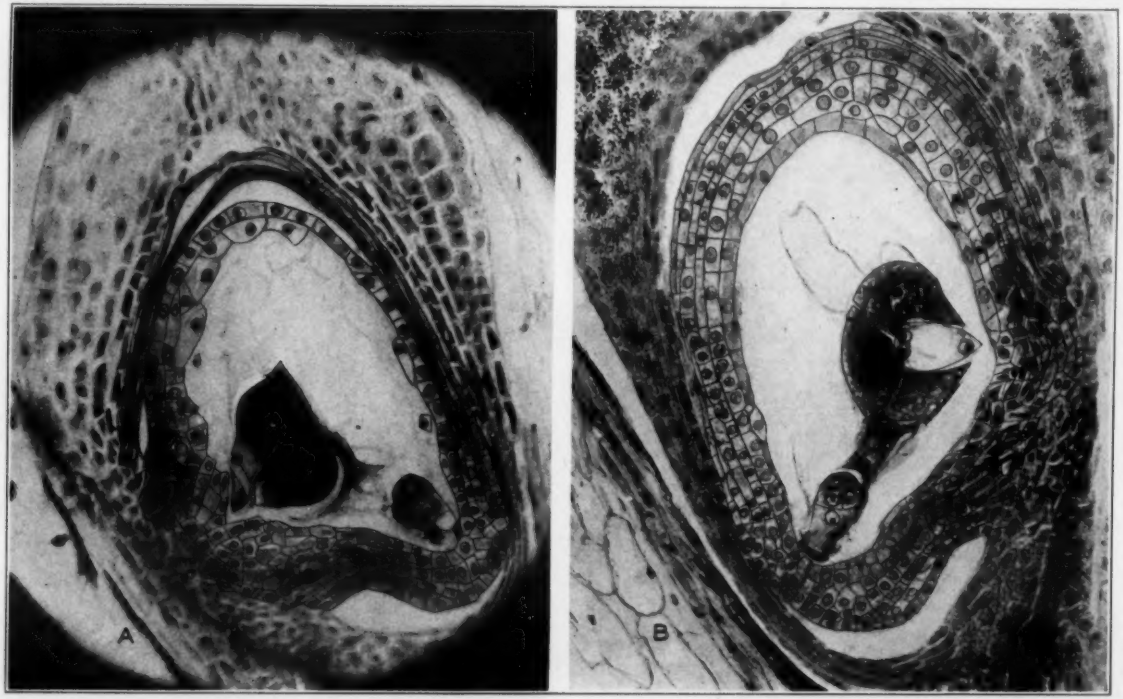
At maturity the two embryos are not always equally developed. Sometimes one embryo is mature while the other embryo is only about one-half grown (pl. 8, B).

Nishimura (*23*) states that in *Poa pratensis* several embryos may arise from a massive suspensor and embryonic buds may also develop from the antipodal nucellar region, and that embryos appear less commonly from the nucellar tissue in other regions of the embryo sac. The writer has observed in sections of several grains the seed entirely filled with endosperm surrounded by the aleurone layer, with one mature embryo at the micropyle and with another embryo about

EXPLANATORY LEGEND FOR PLATE 5

A.—*Poa pratensis*. Photomicrograph of a longitudinal section of an immature ovary, showing an embryo of 3 or more cells at the micropyle, 2 polar nuclei, and 4 or 5 antipodals near the chalaza. × 220

B.—*Poa pratensis*. Photomicrograph of a longitudinal section of a young ovary, showing 2 embryo sacs within the same nucellus. One embryo sac contains an embryo of 5 or more cells at the micropyle, endosperm nucleus, and 3 large antipodals near the chalaza. A four-nucleate embryo sac near the chalaza is surrounded by the antipodals of the other embryo sac. × 220



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(For explanatory legend see p. 1007)

one-half grown outside the aleurone layer. The writer has never found any indication that this is a nucellar embryo. In contrast to Nishimura's interpretation, it is assumed from observations of earlier stages (pl. 3, B, and pl. 7, B) that the embryo outside the aleurone layer is an embryo from a second embryo sac which has been pushed to the side by the growing embryo and endosperm situated more closely to the vascular system.

In germinated seeds showing more than one embryo, each embryo is complete with its own scutellum, plumule, and root. No embryos were observed showing two plumules associated with one radicle or two radicles associated with one plumule.

DEVELOPMENT OF THE EMBRYO

The homologies of the scutellum, epiblast, and plumule sheath of the grass embryo have been much discussed. Kennedy (*16*) gives an extensive review of the work done by earlier authors such as Sachs, Bruns, Hofmeister, Richard, Adr. de Jussieu, Malphigi, Mirbel, Van Tieghem, and many others. Worsdell (*32*) and Coulter (*5*) have written more recently on the subject. The homologies of the parts of the embryos of *Poa compressa* and *Poa pratensis* will not be discussed here; only the development of the embryos will be traced.

Before any external differentiations can be detected the embryo is composed of a number of cells (fig. 1, A, B, C). A slight indentation of the front face is the first external differentiation which indicates the development of growing points (fig. 1, D). With further growth of the embryo, one growing point above the original indentation and two growing points below the original indentation are observed. The growing point directly below the first indentation develops to form the vegetative cone. The growing point above the first indentation and the growing point below the vegetative cone are rudiments of tissue which, with further growth of the embryo, unite to form the plumule sheath or coleoptile encircling the plumule (fig. 1, E).

One section of a slightly older embryo shows these two rudiments of the plumule sheath to have grown until they met, thus forming a complete ring of tissue about the plumule (fig. 1, G). In the adjacent section of the same embryo the two rudiments of the plumule sheath are not united (fig. 1, H). This incomplete union of the plumule sheath may account for the small slit or opening described by other writers in the mature embryos of grasses. One section without the other at this stage could very easily lead one to entirely different interpretations of the formation of the plumule sheath or coleoptile. Hanstein (*9, pp. 47–80*) describes the plumule sheath as formed by the meeting of two outgrowths in Brachypodium; Souèges (*27*) figures the plumule sheath as formed similarly in *Poa annua*.

EXPLANATORY LEGEND FOR PLATE 6

A.—*Poa pratensis*. Photomicrograph of a longitudinal section of an immature ovary, showing endosperm formation in which free nuclei lie in a peripheral, cytoplasmic layer. A one-celled embryo is present. × 100

B.—*Poa pratensis*. Photomicrograph of a longitudinal section of an immature ovary, showing two embryos, each with its own endosperm. × 100

C.—*Poa pratensis*. Photomicrograph of a longitudinal section of an immature ovule, showing one embryo with its endosperm filling one-half of the nucellar cavity. The antipodals have apparently been absorbed. Another embryo which is not visible in this section slightly above the first embryo is associated with the cytoplasm present in the upper part of nucellar cavity and four antipodals which are near the chalaza. × 100

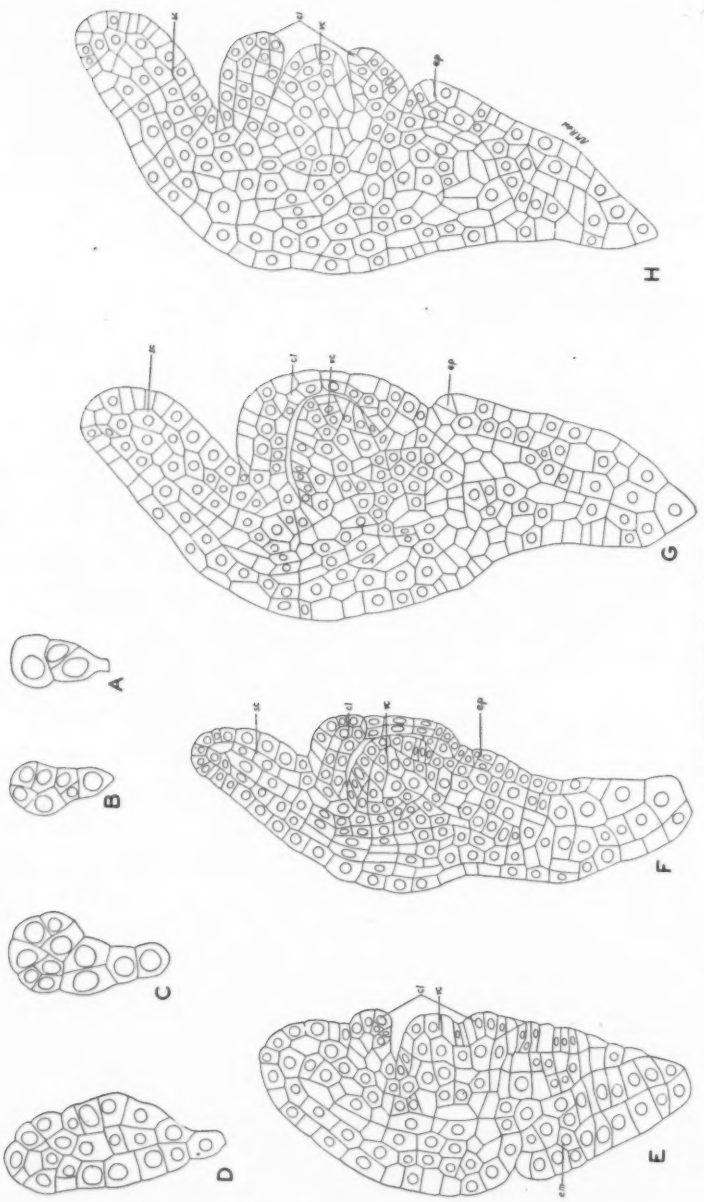


FIG. 1.—(For explanatory legend, see p. 1009.)

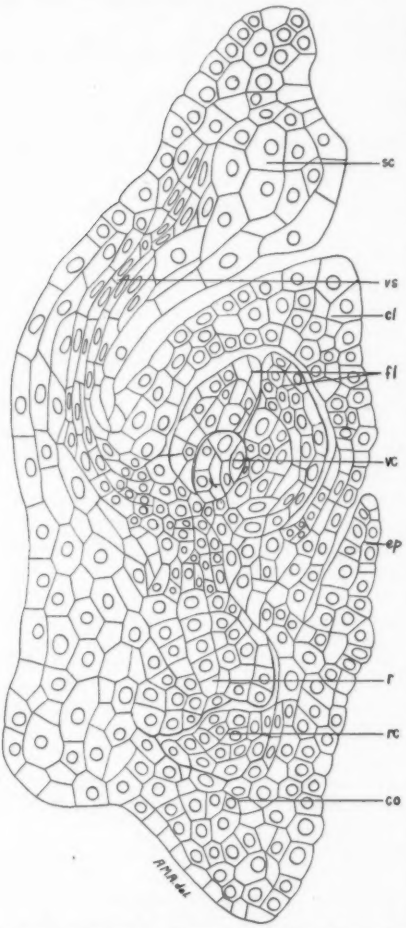


FIG. 2.—*Poa pratensis.* A composite drawing of successive longitudinal sections of a mature embryo, showing scutellum with a poorly defined epithelial layer and with a procambium strand; vegetative cone with the first and second leaves; coleoptile surrounding the plumule; primary radicle terminating with a root cap; the coleorhiza surrounding the radicle and root cap; epiblast. × 280

The epiblast is differentiated at about the time the plumule sheath is formed. The radicle has not been formed at this stage (fig. 1 F).

The mature embryo (fig. 2 and pl. 8, A) in the ripe seed in both species of Poa is situated near the micropyle and lies against the side opposite the chalaza. The scutellum, which is considered the organ of absorption through which the growing parts of the embryo receive their nutriment from the endosperm during germination, is shieldlike in appearance. Near the base of the plumule it is composed of only a few layers of cells but is thicker toward the top. The epidermis, or the layer of cells of the scutellum in contact with the endosperm, does not form a palisade layer as described for some grasses. The plumule has developed into a vegetative cone with two or more rudimentary foliage leaves. Except for a small opening it is surrounded by the coleoptile, or plumule sheath. The primary root is not conspicuously well developed and terminates with a root cap. The radicle and root cap are surrounded by the parenchymatous tissue of the coleorhiza. The primordia of

EXPLANATORY LEGEND FOR FIGURE 1

A.—*Poa compressa.* Longitudinal section of a young embryo composed of 3 cells. × 310
B.—*Poa compressa.* Longitudinal section of a young embryo composed of 9 or more cells. × 310
C.—*Poa pratensis.* Longitudinal section of a young embryo composed of 14 or more cells. × 310
D.—*Poa pratensis.* Longitudinal section of a young embryo, showing indentation which indicates growing points above and below it. × 310
E.—*Poa compressa.* Longitudinal section of an immature embryo. The projection below the first indentation is the vegetative cone. The projection below the vegetative cone and the one above the first indentation are rudiments of the coleoptile. A smaller embryo lies over the lower left half of this embryo. × 310
F.—*Poa compressa.* Longitudinal section of an immature embryo. The coleoptile appears completely to surround the vegetative cone. The epiblast is beginning to be differentiated. × 310
G, H.—*Poa compressa.* Two consecutive longitudinal sections of the same immature embryo. In one section (G), the coleoptile appears completely to surround the plumule. In the following section (H), the lips of the coleoptile are not united. A rudimentary epiblast is present. × 310

For key to abbreviations in figures see page 1002.

secondary roots are not present. A procambium strand leads from the tissue between the plumule and radicle into the upper part of the scutellum. A well-defined epiblast is developed. The epiblast has no vascular strand.

Poa compressa and *P. pratensis* have a complete embryo at maturity. No differences were found in the development of the embryo of these two species which would account for the differences in the physiology of their germination.

INSECT EGGS ON OVARIES OF POA COMPRESSA

When the glumes from the immature pistils of *Poa compressa* were dissected preparatory to a microchemical study of the developing ovule and ovary, a small protuberance was observed on some of the ovaries. It occurs most frequently near the base of the style, although it is found sometimes on the side of the ovary. The ovaries thus affected are lighter in color, somewhat inflated, and slightly larger than the normal green ones. About 20 per cent of the pistils dissected for microchemical studies from material collected at Yonkers, N. Y., showed this minute swelling. Ovaries with protuberances were fixed in Flemming's medium solution, embedded in paraffin, sectioned 12 microns, and stained with Flemming's triple stain.

Examination of the sections showed that these protuberances were not plant tissue but had the appearance of insect eggs. In some cases, one end of the egg extends to a depth of two or three cells in the ovary wall. In one instance, it is partially embedded in the loose tissue beneath the style. In another instance, about one-half of the egg is embedded deep in the tissue of the ovary wall and is in close contact with the outer integument of the ovule.

These slides were examined by G. F. White, of the Bureau of Entomology, United States Department of Agriculture, who observed that the "protuberances" are insect eggs in various stages of development, from a quite early stage to a late one, in which the insect is nearly ready to emerge (pl. 9, A, B, C, D).

From a study of the sections, the shape of the eggs appear to be in general reniform. They range from 84 to 100 microns in width and from 134 to 185 microns in length.

W. A. Riley, head of the department of animal biology of the University of Minnesota, to whom the slides were submitted for further advice regarding classification of the insect, states:

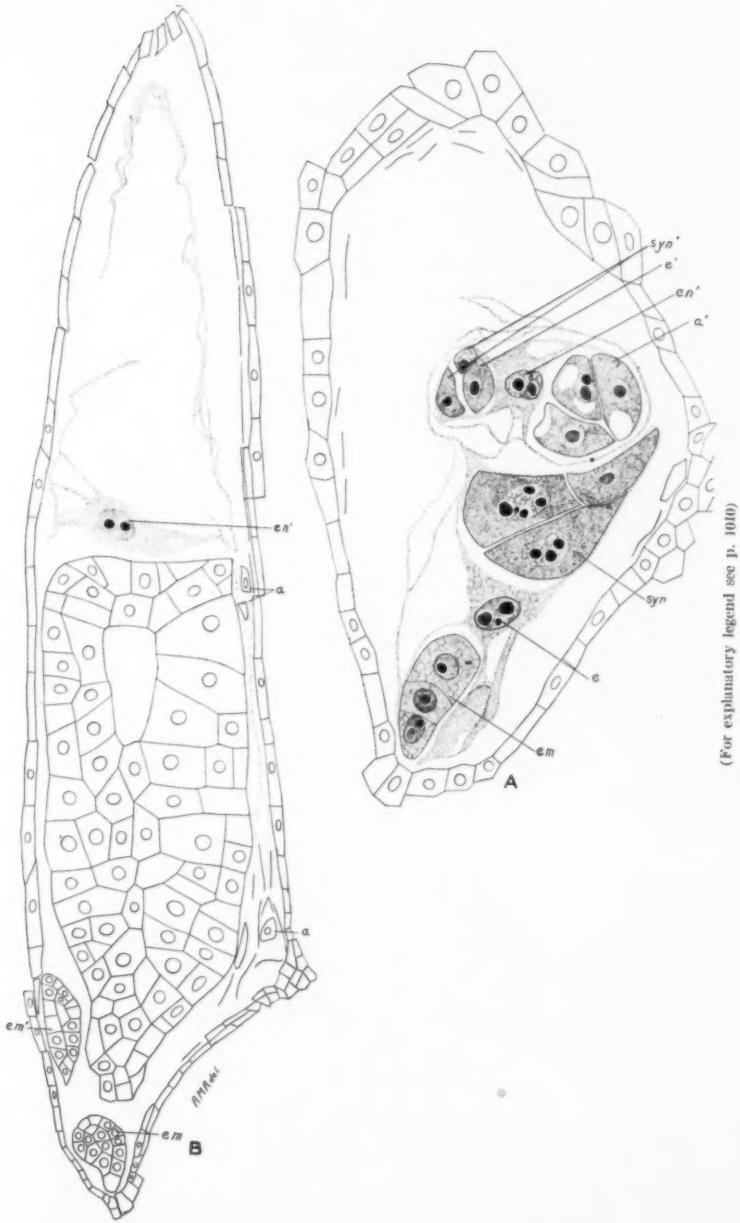
> The insect embryos on the young ovaries of Canada bluegrass which you sent me are those of one of the Thysanophera or 'thrips.' These insects are very common in various flower heads. As far back as 1875 Professor Comstock noted that they were doing great damage to timothy and June grass in New York State. Since that time the species *Anaphothrips obscurus* has been reported from a wide series of grasses, especially in the Northeastern States. It is quite probable that your specimens belong to this same species.

EXPLANATORY LEGEND FOR PLATE 7

A.—*Poa pratensis*. A composite drawing from successive longitudinal sections of an immature ovary, showing 2 embryo sacs within the same nucellus. The embryo sac at the micropyle contains an embryo of 3 or more cells, endosperm nucleus at the center, and 3 large antipodals near the chalaza. The embryo sac farther from the micropyle contains an egg cell and 2 synergids, 2 polar nuclei not fused, and 3 antipodals near the chalaza. × 415

B.—*Poa pratensis*. A composite drawing from successive longitudinal sections of an immature ovary, showing polyembryony. The embryo at the micropyle is associated with the endosperm, filling one-half of the seed. The embryo farther from the micropyle is associated with the undivided endosperm nucleus and cytoplasm pushed toward the distal end of the nucellar cavity. The remains of antipodals are present. × 143

For key to abbreviations, see p. 1002.



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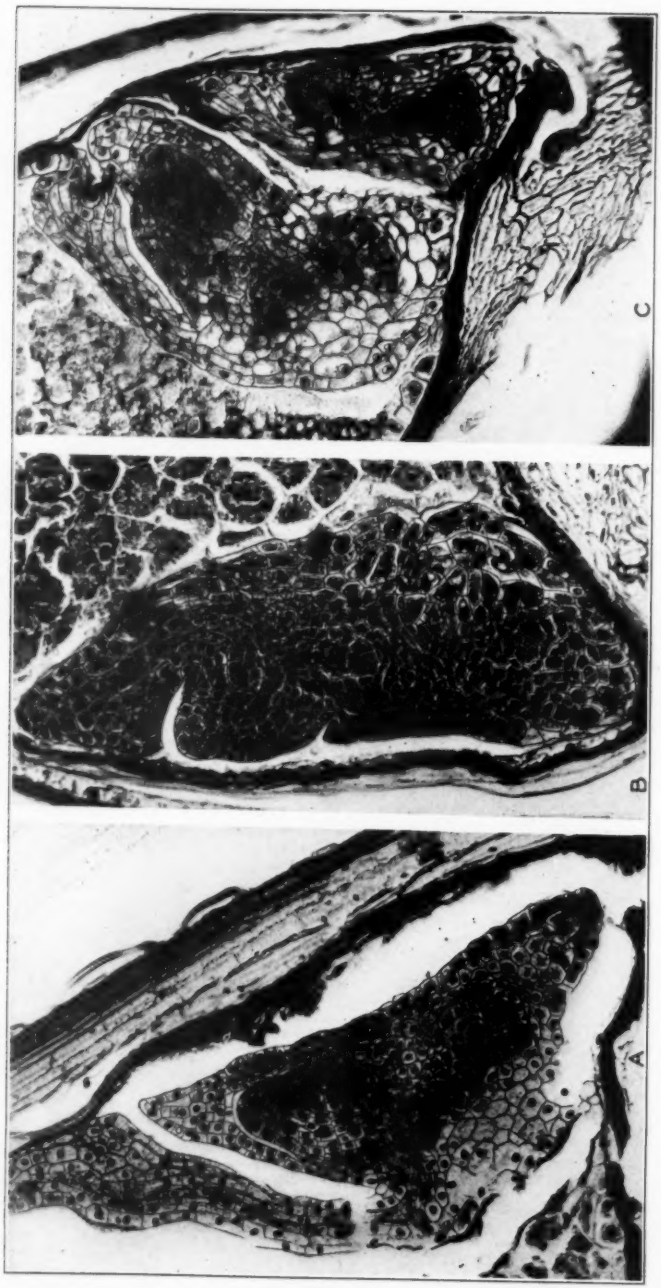

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Osborn (*24, p. 155*), Comstock (*4, p. 127*), Fernald and Hinds (7), Cary (*2*), and others have described the larva, pupa, and adult stages of *Anaphothrips obscurus* Mull. (*A. striatus* Osb.) and its feeding habits. The insect has an incomplete metamorphosis. Fernald and Hinds found that the eggs are deposited in the tissues of the fresh and tender parts of the leaf. The young feed on the head just as it is making its appearance, and upon the juices from the young tender stem inside the sheath. The young insects which enter the top sheath and suck the stem dry above the upper node cause "silver-top." The adults feed upon the leaves and external parts of the grass, and suck the sap from the cells. The empty cells then appear shrunken and white. Fernald and Hinds estimate that in Massachusetts about 40 per cent of the heads of *Poa compressa* are injured with "silver-top." They cite many other hosts. Cary found that in the early months *Anathothrips striatus* confines itself chiefly to June grass, *P. pratensis*, and that as the season advances it is found quite abundantly in timothy, *Phleum pratense*, and on several species of Panicum, Agrostis, and Festuca.

Nishimura (*23*) states that occasionally slightly larger pistils than normal ones were found. He says:

> These were characterized by protuberances. This type of pistil may be due to the deposit of eggs by an insect * * *. Apparently polyembryony and other irregularities are associated with abnormal ovaries. The abnormality arises at different periods in the development of the ovule. In some cases it is associated with the growth of the megaspore, which is seen to become greatly enlarged. Its nucleus at first increases decidedly in size, and this is followed by a massing of the chromatic material, and then its final dissolution. Such megaspores never divide and finally appear as empty sac, often quite equaling in size that of the normal embryo sac * * *. These manifestations of abnormality appear to be associated with the sting of insects which deposit their eggs in the ovary.

In her study of polyembryony the writer has not found an insect egg on an ovary of *Poa compressa* or *P. pratensis* containing two embryo sacs which with further development would have given rise to two embryos. She does not agree with Nishimura, therefore, that insect eggs are associated with polyembryony. Furthermore, from a study of the material at hand the writer is inclined to the belief that some of the ovaries on which insect eggs are deposited do not develop.

DEVELOPMENT AND MICROCHEMISTRY OF THE MEMBRANES OF THE CARYOPSIS [4]

The development of the caryopsis of *Zea mays*, of *Triticum vulgare*, and of *Avena sativa* has been studied by True (*30*). The writer has found no literature referring to the smaller caryopses.

[4] The writer wishes to express her appreciation to Sophia H. Eckerson for methods and suggestions in the microchemical work and to the Boyce-Thompson Institute and the Botany Department of the University of Wisconsin for the use of their laboratories and equipment while this work was being done.

EXPLANATORY LEGEND FOR PLATE 8

A.—*Poa pratensis*. Photomicrograph of a longitudinal section of a mature embryo, showing scutellum, fibrovascular strand, vegetative cone, coleoptile, radicle, root cap, and coleorhiza. The epiblast is differentiated but not distinguishable in photograph. × 200

B.—*Poa pratensis*. Photomicrograph of a longitudinal section showing polyembryony. One embryo is completely developed. The other is only partially developed and crowded directly beneath it. × 210

C.—*Poa pratensis*. Photomicrograph of a longitudinal section of a ripened seed, showing polyembryony. The two embryos are almost equally developed. × 180

The material used for the study of the development of the caryopses of *Poa pratensis* and *P. compressa* was collected in the vicinity of Madison, Wis., in the summer of 1923, and at Yonkers, N. Y., in the summer of 1924. Living plants with the panicles in the different stages of development were brought into the laboratory and placed in water. The ovaries were sectioned with a freezing microtome or placed directly in paraffin and sectioned with a rotary microtome. Microchemical tests of the membranes were then made on these sections. The methods used in the microchemical work were those suggested by Eckerson,[5] Tunmann (*31*), and Molisch (*21*).

Both species of Poa have a simple ovary containing a single ovule (pl. 1, A). The ovary wall is of irregular thickness, being thicker at the top. It consists of an outer epidermis and four or five rows of parenchymatous cells containing much starch. The inner row of cells of the ovary wall consists of chlorophyll-bearing cells, which give the characteristic green color to the young ovary. When the ovule has reached the four-megaspore stage, it is campylotropous. The ovule is attached to the side of the ovary with the micropyle toward the bottom. The integuments do not completely surround the nucellus at this stage. Later the inner integument surrounds the nucellus completely. Each integument is composed of two layers of cells, except that at the base there are sometimes three layers of cells. Both integuments contain starch. At about the time of fertilization the cell walls of the inner integument, adjacent to the nucellus become slightly suberized (fig. 3, A).

At the stage when the embryo is very small and undifferentiated, little or no endosperm has been formed and the antipodals are very large (fig. 3, B), the cell walls of the ovary wall, of the inner and outer integuments, and of the nucellus contain pectic substances. The cell walls of the inner integument adjacent to the nucellus and the cell walls of the outer integument adjacent to the inner integument are suberized. Usually the suberized layer of the inner integument develops earlier than the suberized layer of the outer integument. The walls of the inner cells of the lemma and palea contain cellulose. No lignin is found at this time.

A little later (fig. 3, C, D), when the aleurone cells are differentiated, but still have thin walls, and the caryopsis is still green, the cell walls of the ovary wall and of the aleurone layer contain pectic substances. The cell walls of the ovary wall, and of the lemma and palea give a cellulose reaction. The suberized layer of the cell walls of the inner integument adjacent to the nucellus has thickened somewhat but not as much as the suberized layer of the inner cell walls of the outer integument adjacent to the inner integument. The protoplasm and unsuberized cell walls of the outer integument begin to collapse at about this stage. They are presumably being dissolved and will leave only the thick layer of suberin. In the earlier stages several cases have been observed where all the cell walls of the outer integument were slightly suberized. It may be that these cell walls also persist as thin suberized layers uniting to form the heavy band of suberin. The nucellus, except for one or two cell layers, has been digested and absorbed at this stage.

[5] Mimeographed outlines of Methods of Microchemistry.

Photomicrographs of longitudinal sections through young ovaries of *Poa compressa* and the eggs of *Anaphothrips obscurus*, in different stages of development

A.—Egg in early stage, containing much yolk material. × 100
B.—Egg, showing much yolk material and a developing embryo at one end. × 100
C.—Egg in a later stage of development than B. × 100
D.—Egg, showing insect almost ready to emerge. × 100

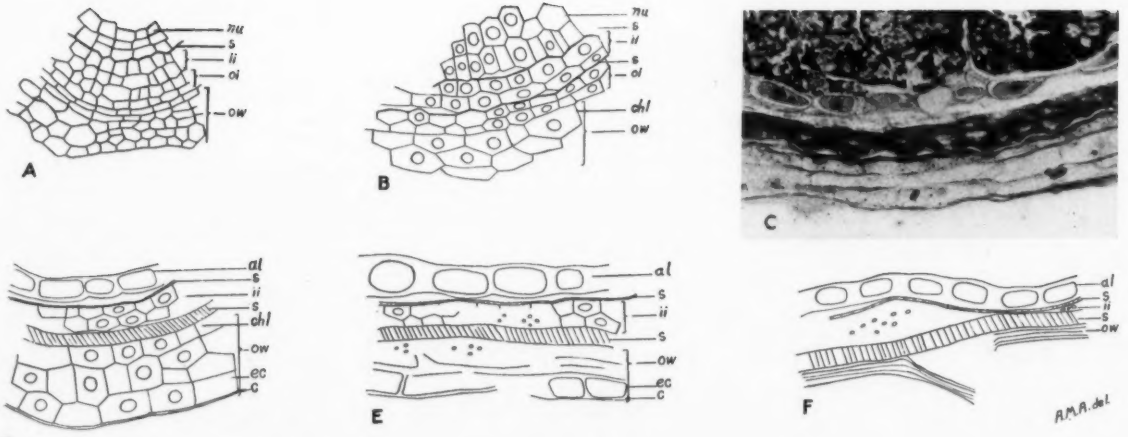

FIGURE 3

A.—*Poa pratensis.* A portion of a longitudinal section of a very young ovule, showing nucellus; inner integument composed of 2 rows of cells and its inner wall slightly suberized; outer integument composed of 2 rows of cells; ovary wall of about 5 rows of cells, of which the inner layer is the chlorophyll layer. × 310

B.—*Poa pratensis.* A portion of a longitudinal section of a young ovule, showing nucellus; 2 integuments, of which the inner layer of the inner and of the outer integuments are slightly suberized; and a part of the cells of the ovary wall. × 310

C.—*Poa compressa.* Photomicrograph of a portion of a longitudinal section of an immature ovule, showing aleurone cells with thin walls; 1 or 2 rows of nucellar cells; inner walls of inner integument suberized; a layer of suberin as the only remnant of the outer integument; ovary wall. × 310

D.—*Poa compressa.* A portion of a longitudinal section of a slightly older ovule than C, showing aleurone cells with thin walls; inner walls of inner integument suberized; a layer of suberin as the only remnant of the outer integument; ovary wall with the epidermis slightly cutinized. × 310

E.—*Poa compressa.* A portion of a longitudinal section of an ovule almost mature, showing aleurone cells with thick cell walls; inner integument with its inner walls suberized, cells less turgid, somewhat shrunken and collapsed, containing some starch; ovary wall cells beginning to collapse. × 310

F.—*Poa compressa.* A portion of a longitudinal section of a mature ovule, showing aleurone cells with thick walls; cells of inner integument compressed, and inner layer suberized; outer integument only a thick layer of suberin; ovary wall cells collapsed and compressed into a thin layer. × 310

For key to abbreviations, see page 1002. Drawings made from sections of fresh material.

At a later stage, (fig. 3, E) when the caryopsis is fully grown, but green, and when the cell walls of the aleurone cells have become thicker, both they and the cell walls of the ovary wall contain pectic substances and cellulose. At this stage the suberized layer of the outer integument has become a very thick layer. This heavy suberized layer is all that now remains of the outer integument. The inner cell walls of the inner integument are suberized as in the previous stage. Their cells are not dissolved and do not disappear but they are somewhat collapsed. The cell walls of the ovary wall also are beginning to show signs of collapse. The walls of the inner cells of the lemma and palea give a cellulose reaction. The rachilla and the walls of the outer cells of the lemma and palea give a lignin reaction.

When the caryopsis is fully grown and mature (fig. 3, F), the cell walls of the aleurone layer, ovary wall, and lemma and palea give a cellulose reaction and also contain pectic substances. The cell walls of the inner integument adjacent to the nucellus form a comparatively thin layer of suberin. The two rows of cells of the inner integument have collapsed somewhat, forming a narrow layer against the suberized layer of the outer integument. A layer of suberin is all that remains of the outer integument. The suberized layers of the inner and outer integuments are so tightly compressed that their identity can be seen only where the seed coat is torn in sectioning. They then appear as two distinct layers, the outer being much thicker than the inner. The pericarp with its characteristic inner chlorophyll layer has also collapsed and forms a thin layer of about the same thickness as the suberin layer. The chlorophyll layer is no longer green, but brown. It gives to the caryopsis a brown color just before maturity. A few starch grains and chloroplasts are still scattered throughout the pericarp. The outer walls of the cells of the epidermis of the ovary wall are slightly cutinized. In the mature caryopsis the pericarp and the integuments lie in close contact and can not be separated readily. The placenta, the nucellus just beneath the embryo, and the walls of the outer cells of the lemma and palea are lignified. The lemma and palea adhere very closely to the ripe caryopsis.

Up to the present time no differences have been found in the morphology or microchemistry of the membranes of the caryopses of these two species, which would account for the differences in the physiology of their germination.

TABLE 1.—*A summary of the microchemistry of the membranes of the caryopses of Poa pratensis and P. compressa*

Stage of development of the caryopsis	Where various substances were found in caryopsis at different stages of development			
	Pectic substances in cell walls of—	Cellulose in cell walls of—	Suberin and cutin	Lignin
1. Three large antipodals and a very small embryo.	Ovary wall, inner and outer integuments, and nucellus.	Inner cells of glumes.	Inner cell walls of inner and outer integuments slightly suberized.	
2. Caryopsis fully grown but green; aleurone cells formed but having thin walls.	Ovary wall and aleurone layer.	Ovary wall and inner cells of glumes.	----do----	
3. Caryopsis fully grown but green; aleurone cells having thick walls.	----do----	Ovary walls, aleurone layer, and inner cells of glumes.	Inner cell walls of inner integument have formed a comparatively thin layer of suberin. Inner cell walls of outer integument heavily suberized. Outer cell walls of epidermal cells of ovary wall are cutinized.	Rachilla and cell walls of outer cells of lemma and palea.
4. Caryopsis mature; ovary wall compressed and shrunken.	Ovary wall, aleurone layer, and glumes.	Ovary wall, aleurone layer, and glumes.	Inner cell walls of inner integument have formed a comparatively thin layer of suberin. Inner cell walls of outer integument are heavily suberized. Outer cell walls of epidermal cells of ovary wall are cutinized.	Rachilla; cell walls of outer cells of lemma and palea; nucellus just beneath embryo; placenta.

SUMMARY

In *Poa pratensis* and *P. compressa* the ovary wall is composed of four or five rows of parenchymatous cells the innermost row of which is the chlorophyll layer, which gives the characteristic green color to the ovary. The ovule is campylotropous. The inner and outer integuments are each composed of two layers of cells.

Four megaspores are formed. Usually the deepest but sometimes the outermost megaspore develops to form the embryo sac. Two megaspores often develop within the same nucellus, and each forms an embryo sac.

The usual eight-nucleate embryo sac is formed.

The antipodals are very large and persist until late in the endosperm formation when they become vacuolated and disappear. Usually three and not more than five antipodals have been observed in one embryo sac. If two embryo sacs are present within the same nucellus six or more large antipodals are present.

The endosperm nucleus divides and forms free nuclei in a peripheral layer of cytoplasm in the nucellar cavity. A parietal layer of cells is formed. The cells later divide and fill in the central cavity. The single layer of aleurone cells is differentiated when endosperm formation is practically complete.

The evidence presented indicates that polyembryony in *Poa pratensis* and *P. compressa* originates from two or more embryo sacs within the same nucellus. In some instances both embryos are

completely developed at maturity; in other instances one is fully developed and the other only partially developed. When two embryo sacs are within one nucellus, a peripheral layer of endosperm is associated with each embryo. Usually only one endosperm surrounded by the aleurone layer has been observed at maturity.

In the development of the embryo, growing points are found in succession. These are the primordia of a vegetative cone, coleoptile, and epiblast.

The coleoptile or plumule sheath is formed by the meeting of two outgrowths about the vegetative cone.

The primary radicle is not differentiated until the vegetative cone and coleoptile are well formed.

The mature embryo has a shieldlike scutellum with a poorly developed epithelial layer. The vegetative cone with its first and second leaves is surrounded by the coleoptile except for a small opening. The primary radicle terminates with a root cap; both the primary radicle and the root cap are surrounded by the coleorhiza. A procambium strand leads from the tissue between the plumule and the radicle into the upper part of the scutellum. An epiblast is present.

In the young ovaries of *Poa compressa* and *P. pratensis*, the cells of the ovary wall contain much starch. Their cell walls give a pectic reaction but later also contain cellulose. The outer cell walls of the epidermal cells become cutinized as the seed matures.

The cells of the inner and outer integument contain much starch. Soon after fertilization a layer of suberin is found in the cell walls of the inner integument adjacent to the nucellus, and in the cell walls of the outer integument adjacent to the inner integument. Usually the inner suberized layer develops first. With further development of the caryopsis these suberized layers increase in thickness. The outer one becoming thicker than the inner. The protoplasm and unsuberized cell walls of the outer integument disappear and are presumably absorbed. The cell walls of the inner integument begin to collapse by the time the caryopsis has reached its full size.

The cell walls of the young glumes contain cellulose. As the seed reaches maturity the cell walls also contain lignin and pectic substances.

The mature caryopsis consists of a fully developed embryo and endosperm surrounded by a single layer of aleurone cells which have thick walls, one or two rows of collapsed nucellar cells, modified integuments, and pericarp. The inner layer of the inner integument consists of a comparatively thin layer of suberin. Its remaining cell walls and their contents are compressed. The inner layer of the outer integument adjacent to the inner integument is composed of a thick layer of suberin. The remaining cells and their contents are presumably dissolved. The ovary wall cells are compressed into a thin layer. The outer cell walls of the epidermis are cutinized. The lemma and palea which adhere closely to the ovary contain lignin and pectic substances.

No differences have been found in the development of the embryo or of the membranes of the caryopses of *Poa compressa* and *P. pratensis*, which would account for the differences in the physiology of their germination.

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LIFE HISTORIES AND HETEROTHALLISM OF THE RED BREAD-MOLD FUNGI OF THE MONILIA SITOPHILA GROUP[1]

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INTRODUCTION

The red bread mold has long been known as a bakery pest and has caused much loss to bakers as well as to housewives. Its occurrence is somewhat erratic, but when a bakery becomes infested there is more or less continual trouble and loss until the fungus has been thoroughly eradicated.

Related species develop extensively on sugar-cane bagasse and this becomes a serious problem where this bagasse is used commercially as a basis for insulating board. The fungi of this group have also been found on silage and sometimes in connection with field, transportation, and storage rot of fruits, especially strawberries, raspberries, and apples, but appear to be of little economic importance in such cases. They are also well known to laboratory workers in mycology and pathology as a contamination in cultures of other organisms. The conidia are so light and powdery that they are easily and widely disseminated by the faintest breeze or movement of the air to which they are exposed.

HISTORICAL REVIEW

The common red mold appears to have been first reported in scientific literature by Léveillé (*37, p. 5, 6, 8*)[2] in France in 1843 under the name *Oïdium aurantiacum.* The same year it was also described by Montagne (*35, p. 377–378*) as *Penicillium sitophilum.* It was finally transferred to the genus Monilia by Saccardo. The fungus was called to the attention of Léveillé and Montagne by a commission (*37*) which was appointed by the French Minister of War in 1842 to investigate the cause of an infestation of the army bakeries which destroyed large quantities of bread. Another outbreak of the mold in bakeries in France was reported in Paris in 1871 by Decaisne (*9, 10*). De Claubry (*18*) also investigated this case and says that he had seen the same thing in bread in France in 1831. Decaisne states that sickness in one family whose members ate the moldy bread was attributed to the mold, but no evidence was found to support this. Outbreaks of the same fungus in bakeries have been reported by Murtfeldt (*36*) in Germany in 1917, by Mattirolo (*32*) in army bakeries in Italy in 1918, and by Herter (*20*) in Berlin in 1919. The same trouble is frequently reported from bakeries in this country, and only a few weeks ago cultures of the fungus were received from two different bakeries in New York.

[1] Received for publication Feb. 3, 1927; issued July, 1927.

[2] Reference is made by number (italic) to "Literature cited," p. 1040.

In connection with the investigations of this fungus made in France, L. R. and C. Tulasne (*49, p. 90*) give an interesting account of its temperature relations, which being translated reads as follows:

> Mauduyt of Poitiers, an expert in all branches of the natural sciences, in whose company we received our first knowledge of them, once thought that the dust [spores] of *Oidium auranticacum* Lév. (*Penicillium sitophilum* Mont. in Ann. Sci. Nat. ser. II, t. xx, 1843, p. 377, pl. 16, f. 4, and Syllog. Pl. Crypt. 1856, p. 301), when mixed with ground meal and then baked in a hot oven, was damaged, so that if the bread afterwards went bad he thought the reason was to be found not at all in the included dust but in spores fallen from the air. He also tested some bread of the previous day which was everywhere much defiled by the *Oidium*, and when he had boiled it in water for half an hour it was never again spoilt by the contained mucedinous fungus or covered afresh by the red powder, but a piece of the same bread, when purified by similar boiling, could be readily infected by the seeds of *Oidium* falling from the air or purposely scattered over it, so that within two days it became completely red. (*Cf. Epist. ad Pictonum ediles* missam, Oct. 30, 1847.) The recent experiments of the famous L. Pasteur on the Mucedinei agree with the statements of the Poitevin naturalist. For they prove that the same spores of Mucedines, which could withstand without harm a heat of 120° or 125° for one hour or more in dry air or in a tube exhausted of air, were killed at once in boiling water; and the same thing happened with the dusty particles floating in the air, to which, falling perpetually, that author thinks all kinds of fermentation are due. (*Cf. Comptes Rendus*, t. LII., p. 16–19, Jan. 7, 1861.) All this agrees with what Turpin says about *Torula Cerevisiae* Turp. (in Walter Claubey's *Repert. Chim.* t. for 1838, p. 272, 279), for that fungus, as shown by Colin and Thénard, when placed for a little while in boiling water, lost at least for a time thereby any power of exciting the fermentation of a sugary liquid. Therefore the mode of action of the Gascons is seen to be contrary to the opinions of the learned, and we believe the only experiments on which reliance can be placed are those by which Payen asserted he had proved that the seeds of *Oidium auranticacum* Lév., even when moistened retained their power of germinating uninjured, after being subjected to a temperature of 105° and 120°, so that the experimenter need not be surprised if bread made of contaminated wheat soon goes red on account of the Mucedine which it contains, since the inside of the loaf is not thought to be exposed in the oven to a heat of 100°. (*Cf. Comptes Rendus*, t. xxvii, p. 4–5, July 3, 1848.)

The mold in loaves of bread develops from spores contaminating either the flour after it has been milled or the wheat before it is ground. After the mold has been introduced into a bakery the dough from which the bread is made may also be contaminated by spores floating in the air of the bakery. According to recent experiments made by Tokugawa and Emoto (*48*), conidia of *Monilia sitophila* are killed in saturated steam at 100° C., although in a moist condition they are able to survive a temperature between 70° and 80° for five minutes, and in a dry atmosphere they are able to endure a temperature as high as 130°. There would be little probability, according to the statements of the authors just cited, of the conidia being destroyed in the loaf in baking, since records of baking temperatures made by the Bureau of Home Economics of this department show that when bread is baked in an oven at a temperature of 200° C. the temperature of the interior of the loaf does not exceed 95° to 98°. According to the temperature tests of the writers, conidia immersed in water and heated to 72° for four minutes are killed, and most of them are killed when subjected to a temperature of 65° for the same period. Moisture conditions, period of heating, and other factors enter so largely into the determination of the death point that it is difficult to tell whether or not the spores would be killed in ordinary baking.

Various physiological experiments with this mold have shown that it will cause fermentation and produce alcohol in the absence of

oxygen. It has also been demonstrated by Went (*51*, *52*) that it produces 10 different enzymes as follows: Maltoglucase, trehalase, raffinase, invertase, cytase, diastase, lipase, tyrosinase, labenzym, and trypsin. Kunkel (*27*, *28*) made a study of the toxicity of various inorganic salts upon the growth of this fungus. He found that of various nitrates tested potassium nitrate is least toxic and zinc most toxic. He also found that toxicity measurements made without regard to the inorganic substances in the culture medium are of little value.

In none of the literature relating to this fungus that the writers have been able to find is there mention of the finding of perithecia of *Monilia sitophila* either in nature or in artificial cultures. Went (*51*) in his physiological studies describes and illustrates the beginning of sclerotoid bodies resembling young perithecia. His only reference to them, however, is in the explanation of the plate in which he calls them "Hyphenknauel." They may have been the fundaments of sterile perithecia.

In 1901 Alfred Möller (*34*, *p. 294*, *pl. 2*, *fig. 34*), in his studies of tropical fungi, described a red mold found on corn bread and carbonized tree trunks, which he named *Melanospora erythraea*. In growing this fungus in culture he obtained an orange-colored mold which he did not attempt to name, but which according to his description and illustrations taken in connection with the writers' recent studies of the life history *of Monilia sitophila* was undoubtedly very closely related to this species, since the perithecial as well as the conidial forms are very similar. No one seems to have recognized heretofore the close relationship of Möller's conidial fungus to the common *M. sitophila*.

In 1924 Kitasima (*24*, *25*), of Japan, published a report of observations which he had made upon an orange-colored mold found growing abundantly on trunks of trees which were burned during the great fire in Tokyo. He referred the fungus to *Monilia aurea* Gmel. Growing this in culture he obtained perithecia and ascospores of a fungus which he referred to the genus Anthostomella. An examination of Kitasima's fungus, which he sent the writers, shows that his conidial stage can not at present be distinguished from the common *M. sitophila*, and that the perithecial form, which he called Anthostomella, is really more nearly related to Melanospora as generally interpreted, and is evidently the same as that which the writers have obtained from *M. sitophila*.

LIFE HISTORY AND TAXONOMIC STUDIES

A few years ago Charles Thom, of the Bureau of Chemistry, in studying the organisms found on sugar-cane bagasse obtained cultures of a Monilia which produced both conidia and perithecia. Thom and Lathrop (*47*) in referring to this fungus say: "On the surface of the bales the first organism to develop was *Monilia sitophila*, which spread over the piles with great rapidity and draped them with festoons of orange-fruiting masses. Even after three to four weeks, handfuls of mycelium and spores could be gathered in many places." Cultures of this fungus furnished by Thom and Miss V. K. Charles, of the Bureau of Plant Industry, were studied and single ascospore cultures obtained which produced conidia very similar to typical *M. sitophila*.

A little later several cultures labeled *Monilia sitophila* were obtained from Westerdijk's culture collection at Baarn, Holland. One of these had been supplied by Stahel, of Dutch Guiana, another came from Herter, of Berlin, and another was originally grown by Bainier, of France. Much to the surprise of the writers, the culture from Dutch Guiana developed many perithecia, which were very similar in character to those in the cultures from Japan and Louisiana. Careful comparison, however, of perithecia, ascospores, and conidia shows that there are specific differences between them. These differences are shown by the descriptions and illustrations which follow.

A part of Möller's type of *Melanospora erythraea*, sent by H. W. Wollenweber, of Berlin, shows that it is different from the Surinam plant which the writers had at first thought might belong to the same species.

The association in cultures of conidia similar to those of *Monilia sitophila* and perithecia, which were referred by Möller (*34*) to Melanospora and by Kitasima to Anthostomella, naturally suggested a genetic relation between the two spore forms. In fact Kitasima (*25, p. 17*) says: "I have succeeded to get a pure culture derived from a single ascospore, produced the conidia which are of Monilia type in coloration, size, and shape." He does not state that perithecia appeared in this culture. The writers do not find, however, that anyone has reported the production of good perithecia in monosporous cultures of either conidia or ascospores and their early attempts were equally unsuccessful. This led naturally to the suggestion that the writers were dealing with a group of heterothallic species.

First efforts to grow single ascospore cultures were largely unsuccessful, owing to failure of the ascospores to germinate under ordinary conditions. It was soon found, however, that heating the spores caused them to germinate more readily. By pairing a large number of different ascospore or conidial cultures from different sources, it was demonstrated that certain paired cultures produced perithecia in abundance while others produced none. It was thus demonstrated that of the three species with which the writers have worked two are heterothallic, i. e., consist of two sexually different haplonts, and one species is homothallic. A full account of this phase of the work will be given in connection with the discussion of culture studies.

The discovery of another spore form of this mold may have important bearings upon the means of dissemination and the chances of reproduction of the fungus, and hence of its prevention and control. It is also important to know that there are several species which may infest bread, fruit, and other plant products and that they are heterothallic. If only one sex is present, the fungus should be easier to eradicate when it is found in a bakery or any other place, as no ascospores would be produced.

In attempting to determine the identity and systematic relationships of these species it was necessary to compare them carefully with the genera having similar perithecial forms as well as conidial stages. In the present state of our knowledge of the Pyrenomycetes it is difficult to define accurately and to limit the various genera that have been described. As at present treated, many genera include a heterogeneous group of species. It is therefore desirable to select some particular species of a genus as a basis for a concept of the group.

As already mentioned, two of the species with which the writers are concerned have been referred to different genera. Möller (*34*) referred his species to Melanospora, and Kitasima (*24*) referred his to Anthostomella, the genus to which *Melanospora destruens* (Shear) comb. nov. was first referred.

Melanospora was described by Corda (*7, p. 24*) in 1837. He included in it three species in the following order: *Melanospora zamiae*, *M. chionea*, and *M. leucotricha*, all of which he illustrated. The first species, *M. zamiae*, has not since been reported, as far as known, except for a specimen found on rice in India and so named by E. J. Butler. The other two species are very similar and apparently congeneric. The life history of neither of these is satisfactorily known. Zukal (*53, p. 538*) grew *M. leucotricha* but obtained no conidia or pycnidia. The ascospores germinated from one end only. In accordance with present usage and in the interest of stability and uniformity in nomenclature it seems best to accept *M. chionea* (Fr.) Cda. (pl. 1, A), the commonest and best known species of Corda, as the nomenclatorial type of Melanospora, *M. chionea* has membranous, gray perithecia, with a long slender beak which is fimbriate at the apex. No conidia are known. The perithecial form of *Monilia sitophila* differs from this in the color and character of the perithecia, in the absence of a distinct elongate fimbriate beak, and in its persistent asci.

Anthostomella, to which Kitasima (*25*) referred the perithecial form of his Monilia, is really less nearly related to this fungus than Melanospora and several other closely related genera. Anthostomella was described by Saccardo (*40, p. 84*). *Anthostomella limitata* Sacc., the first of the three species originally placed in the genus by the author, is apparently a good representative of the group as generally accepted. The perithecia in this species are submembranous, gregarious, entirely embedded in the tissue of the host, and covered with a more or less blackened clypeus or epistroma. Good paraphyses are also present and no conidial form is known.

Ceratostoma of Fries (*15, p. 337*) is another related genus described in 1818 with 35 species. *Ceratostoma* (*Melanospora*) *chionea* was included as No. 16 of the list. In 1849 Fries (*16, p. 396*) revised his idea of the genus and included but six species, specifying particularly *C. chionea* as the type of the genus. In a footnote he makes the following statement (translated):

> When I collected *S. chionea* I immediately considered it to be the type of a new genus=Ceratostoma Obs. Myc. II; then I saw a very close relationship to various beaked Sphaerias which could be in no way distinguished. For this reason I reduced them to Sphaeria in the S. M., and moreover the limits are changing, for Sphaerias No. 110–112 should be placed here with equal justice. Meanwhile following the suggestion of recent authors, I separated this and the following subgenera, at the same time predicting a close connection with Sphaeronaema; for species of this genus having a globose perithecium sharply distinct from a beak can not be distinguished from Ceratostoma. The name Melanospora Cord. is suitable for only the first species, therefore, we have fixed upon the older name to be retained.

It is clear from this that he regarded his genus as a synonym of Melanospora of Corda. The usage of later authors, however, as Fuckel (*17, p. 127*), Schroeter (*42, p. 312*), Saccardo (*41, p. 215*), and Lindau (*29, p. 406*) has changed the application of the name by retaining Melanospora for *M. chionea* and similar species, and

applying Ceratostoma to a group of species of which *C. notarisii* Sacc. may be designated as the nomenclatorial type. In adopting this interpretation Lindau (*29, p. 406*) says in a footnote (translated):

> As Schröter has shown, Fries's concept of the genus does not correspond with the present application of the name. The species he recorded as belonging to it must be referred to Melanospora. If one follows priority *Ceratostoma* must replace *Melanospora*, and a new name be given the present group of species. This would only make confusion. I therefore accept the present usage.

According to this interpretation of Ceratostoma the writers' species differ from it in having neither a carbonous, long rostrate perithecium nor a true persistent paraphyses and in having a Monilia conidial stage.

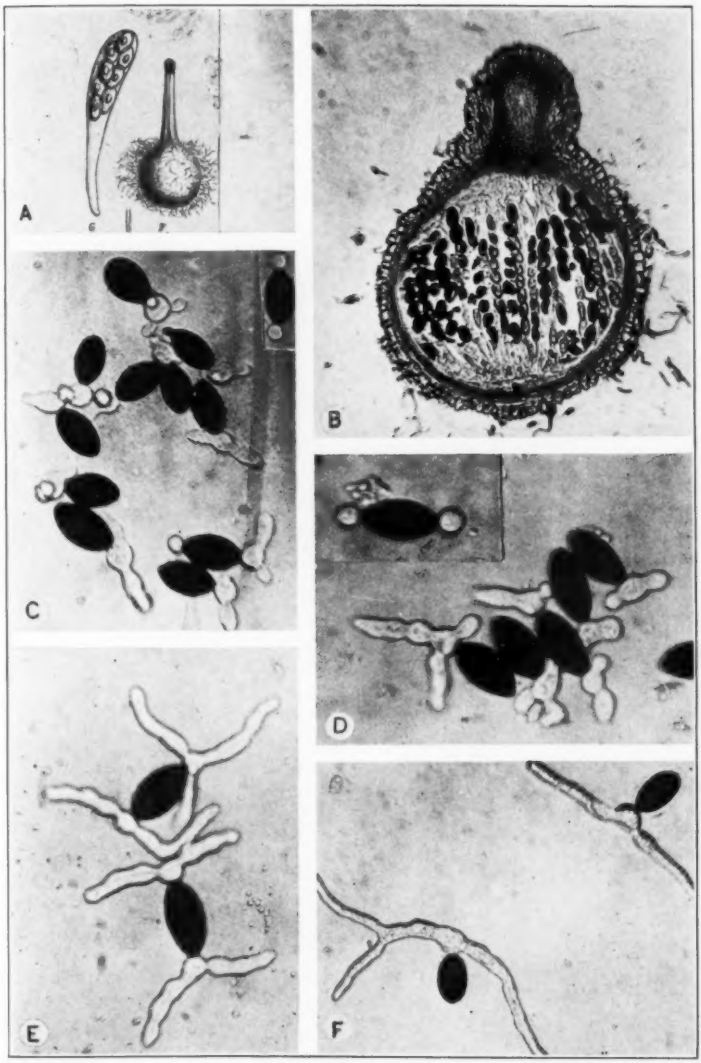
Included in Sordaria and Podospora are certain species which show many points of relationship with the fungi treated here. Some of these species are known to have conidia of the Spicaria type, e. g., *Sordaria montanensis* Griffiths (*19, p. 49, pl. 19, fig. 13*) and *Podospora brassicae* Brefeld (*5, p. 196–198, pl. 6, figs. 1–2*). The same thickened ring is also found in the apex of the ascus in some species of these genera. *Ascotricha pusilla* (E. and E.) Chivers (*6, p. 220, pl. 17*) and some species of Chaetomium also show close relationship.

Certain species referred to Rosellinia, Sphaeroderma, Erythrocarpa and Sphaerodermatella are also nearly related and are in need of further study and comparison before the natural boundaries and relationships of these genera can be determined.

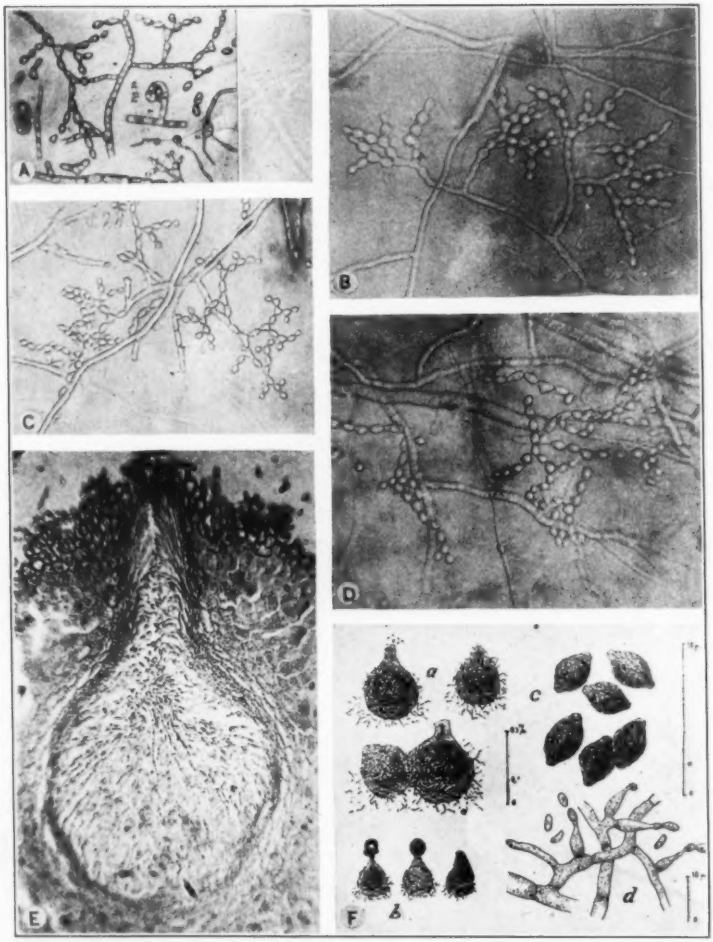
As far as known, the nearest relative of the new genus Neurospora, to which the red bread-mold fungus is being referred, was described by Vincens (*50*) as *Melanospora mangini* (pl. 2, F). This has very similar perithecia and the ascospores have anastomosing ridges. It also has a conidial stage which the author refers to Spicaria (pl. 2, F, *d*). Several other species referred to Melanospora have known conidial forms which are referred to Spicaria. These are evidently more closely related to the new genus Neurospora than to true Melanospora. Whether they should be transferred to this new genus or not must depend upon further knowledge of the systematic value of the ascospore characters and the conidial stage. The conidial forms of other species of Melanospora have been referred to Scopulariopsis and Acmosporium. Another group of species which has been referred to Melanospora may be represented by *M. destruens* (Shear) (*43; 44, p. 43*) (pl. 1, B; pl. 3, D). This group differs from the typical Melanospora as reeprsented by *M. chionea* in having thicker subcoriaceous perithecia and short, nonfimbriate ostioles.

Small globose bodies resembling sclerotia and known as bulbils, which have been found associated with cultures of species referred to Melanospora and are placed in the form genus Papulospora as treated by Hotson (*22*), usually develop in monosporous cultures of the heterothallic species of Neurospora, and sometimes occur in the homothallic species and in the cultures from the paired haplonts. Berkhout (*2, p. 39*) also reports such bodies in her cultures of *Monilia sitophila*. Whether these are the fundaments of sterile or aborted perithecia or a peculiar form of asexual body can not be stated at present.

The genus Monilia, to which the conidial stages of Neurospora are here referred, was first described by Hill (*21, p. 69*) in 1751. In this



Photomicrographs of Melanospora and Neurospora: A, Perithecium and asceus of *Melanospora chionea* (after Winter); B, section of perithecium of *M. destruens;* C–F, germinating ascospores; C. *Neurospora sitophila;* D, *N. crassa;* E, *N. tetrasperma;* F, *Melanospora destruens.* B× 148,C–F × 368



Conidia and perithecia of Neurospora and Melanospora: A, Conidia of *N. erythraea* (after Möller); B, *N. sitophila;* C. *N. crassa;* D, *N. tetrasperma;* E, section of young perithecium of *N. crassa* showing cavity filled with degenerating hyphae resembling paraphyses; F, perithecia, *a, b*; ascospores, *c*, and conidia, *d*, of *Melanospora mangini* (after Vincens). A–D × 185. E × 216

connection it is interesting to note that he makes a statement which it has taken nearly 200 years to verify. His statement is as follows:

> The Monilia all produce distinct male and female flowers. The male flowers are antherae, placed on a short stamina, on the summit of the pedicle, or near the summit, surrounding it in form of a fine powder. The female flowers we are able to distinguish nothing of, except the seeds, which are arranged together in series by means of a glutinous matter, and stand sometimes close to one another, sometimes more distinct.

This was written during the period when the question of sex in flowering plants was being much discussed by botanists. Hill was one of those who having accepted sex in plants thought it should be true of fungi as well as flowers; hence his rather naïve attempt to distinguish male and female flowers in Monilia.

Hill's conception of the genus was based upon Micheli's (*33, p. 212, tab. 91*) descriptions and figures of Botrytis and Aspergillus. The present so-called form genus Monilia is not usually credited to Hill because he was not a binomialist, and because of his broad and too indefinite application of the name.

The name is usually attributed to Persoon (*38, p. 691*), who first used it in 1801 to include 12 species. These were as heterogenous as those of Hill. The original species of Persoon have gradually been separated and most of them placed in other genera. According to present usage, *Monilia fructigena* Pers., the conidial stage of *Sclerotinia fructigena*, might well be taken as the nomenclatorial type of Monilia. If the names of conidial stages were to be restricted to those having the same generic perfect stage it would be necessary to refer *M. sitophila* to another form genus. It does not seem desirable, however, to follow such a course, as the inconvenience and change would be much greater than any benefit which might be derived. Until all the conidial forms have been connected with their perfect stages it will be necessary to retain these form genera to include conidia and pycnidia which have great morphological similarity but different life histories or perfect stages.

The results of the investigations of *M. sitophila* reported here show that the conidial forms of several species of Ascomycetes have heretofore been included under this name. As will be seen from an examination of the descriptions which follow, the differences in the conidia of the different species are comparatively slight, and can be determined only by careful cultural studies made in connection with the perithecial stages. According to our present knowledge the synonomy of *M. sitophila* (Mont.) Sacc. is as follows: *Penicillium sitophilum* Mont.; *Oidium aurantiacum* Lév.; *Monilia martini* Ell. and Sacc.; *Oospora aurantiaca* (Lév.) Herter; *Monilia aurantiaca* (Lév.) Herter not Peck and Saccardo.; *Oidium lupuli* Matth. and Lott. (*31, p. 86–87*); *Oospora lupuli* (Matth. and Lott.) Lindau (*30*); *Monilia aurea* Kitasima not Gmelin; (?) *Monilia carbonaria* Cke.

As the result of the life history and morphological studies of the red bread-mold fungus and its near relatives a new genus, Neurospora, and three new species are described.

Neurospora gen. nov.

Perithecia gregarious or scattered, smooth or with loose hairs, subcoriaceous to subcarbonous; ostiole papillate or short rostrate, perithecial cavity lysigenic, filled at first with parallel, septate hyphae (paraphyses?) which begin to collapse and disappear as soon as the young asci start and are entirely gone when the asci

are full grown (pl. 2, E); spores hyaline to yellowish-brown at first, becoming black or greenish black when mature, continuous, longitudinally ribbed (pl. 3, A). Conidial stage of the *Monilia sitophila* type (pl. 2, B).

The perithecia are either superficial or embedded when grown on agar media; when superficial they usually show a loose growth of weak hyphae on the surface. The crowded septate hyphae which fill the perithecial cavity at the time the asci start are doubtfully described as paraphyses. They disappear so soon that they would not be observed except in very young perithecia.

Neurospora sitophila sp. nov.

Conidial stage (*Monilia sitophila* Mont.): On corn-meal agar tufted or irregularly pulvinate at first, soon becoming effuse and fluffy; conidial masses varying from pale salmon to orange color; mycelium hyaline, creeping, septate; sporogenous hyphae irregularly ascending, septate, dichotomously branched; conidia catenulate, connected by a narrow isthmus when mature, globose to subglobose, smooth, pale salmon to orange in mass, 10 to 12μ diameter, mostly 10 to 11μ (pl. 2, B).

Perithecial stage: Perithecia gregarious or scattered, superficial or immersed on agar media, smooth or loosely soft, hairy, brown to black subcoriaceous, 200 to 300μ diameter; ostiole papillate; asci cylindrical, short stipitate, apex with a thickened gelatinous ring, 8-spored, 140–160$\times$12–14μ paraphyses (?) crowded, multiseptate, filling the perithecium at first, collapsing and disappearing as the asci develop; ascospores overlapping uniseriate, elliptical, with 16 to 17 sometimes branched longitudinal ribs, olivacious becoming dark greenish black, 20–26$\times$10–15μ, mostly 23–25$\times$13–15μ (pl. 2, A; pl. 3, A).

Type: Selected, culture No. 5574, slide No. 5575, from which Plate 4, A, was taken and in mycological collections, Bureau of Plant Industry. Original from Herter, Berlin.

The description is based on cultures on corn-meal agar in test tubes. Whether this species is identical with Montagne's fungus it is impossible to determine at present, as the ascospore stage of his plant was not found. This is the common red bread mold of Europe and America and presumably Montagne's (*35*) species; for practical purposes, therefore, this name may be adopted.

Neurospora crassa sp. nov.

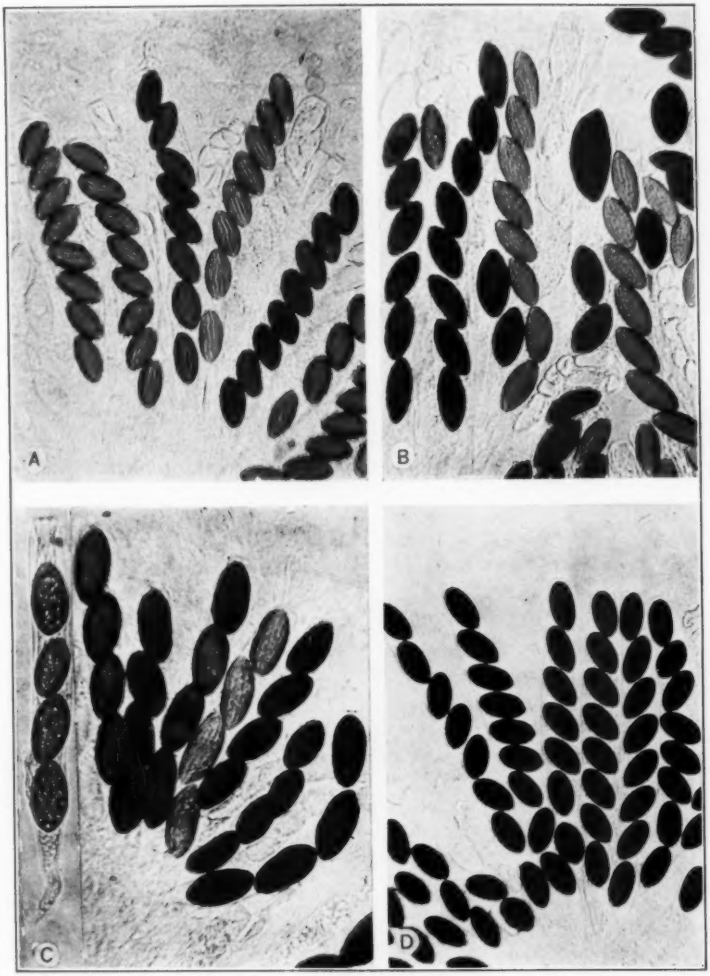
Conidial stage (*Monilia crassa* nom. nov.): On corn-meal agar tufted or irregularly pulvinate at first, soon becoming effuse and forming fluffy aerial masses at the sides of the tube, pale salmon to light orange. Mycelium scanty, creeping, septate; sporogenous hyphae, irregular, ascending, septate, dichtomously branched; conidia catenulate, connected by a narrow isthmus, globose to subglobose when mature, smooth, yellow to pale orange in mass, 6 to 8μ diameter, mostly 6 to 7μ (pl. 2, C).

Perithecial stage: Perithecia gregarious or scattered, subglobose, superficial or immersed when grown on agar, smooth or loosely soft hairy when superficial, yellowish brown at first, becoming blackish brown, thick walled, subcoriaceous, 400 to 600μ diameter; ostiole obtuse, conical, black; asci cylindrical, short stipitate, with a thick gelatinous ring at the apex, 4-spored, 150–175$\times$18–20μ; paraphyses (?) crowded, multiseptate, filling the perithecium at the time the asci appear, collapsing and disappearing as the asci develop, entirely lacking in the mature perithecium; ascospores overlapping-uniseriate, elliptical, with about 20 longitudinal, sometimes branched ridges, olivaceous at first, becoming dark brown to black, 23–32$\times$11–16μ, mostly 27–30$\times$14–15μ (pl. 4, B; pl. 3, B). This species is heterothallic.

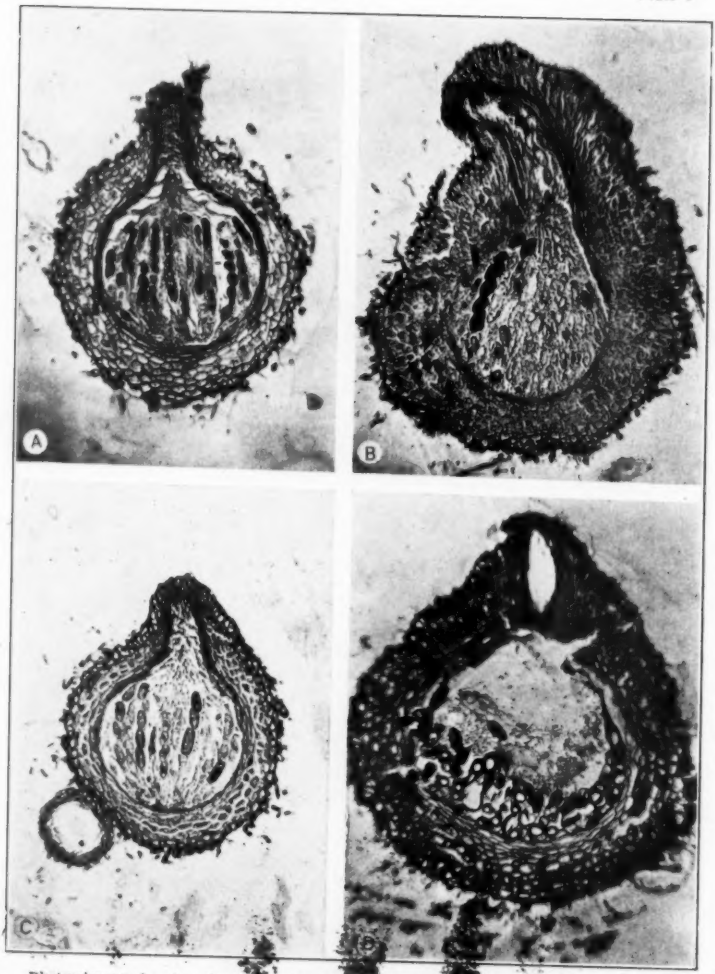
Habitat: On bagasse, Marrero, La. From Charles Thom, of the Bureau of Chemistry. Known only from this material.

Type: Culture No. 5576, slide No. 5577, from which Plate 4, B, was taken. In mycological collections, Bureau of Plant Industry.

On corn-meal agar in Petri dishes the conidial masses form about the sides of the dish and are about the same color as in *Monilia sitophila*, but smaller in quantity than in that species when grown on the same medium and under the same conditions. The same difference occurs in the cultures but is less striking. On bread in flasks the conidial growths are very similar.



Photomicrograph of asci and ascospores of Neurospora and Melanospora: A, *N. sitophila;* B, *N. crassa;* C, *N. tetrasperma;* D, *Melanospora destruens* × 332



Photomicrographs of sections of perithecia of four species of Neurospora: A, *N. sitophila;* B., *N. crassa;* C, *N. tetrasperma;* D, *N. erythracea.* × 126

Neurospora tetrasperma sp. nov.

Conidial stage (*Monilia tetrasperma* nom. nov.): On corn-meal agar in tubes very scanty whitish growth of mycelia about the upper edge of the agar slant; conidial tufts usually small and scanty, pale salmon; sporogenous hyphae irregularly ascending, septate, dichotomously branched; conidia catenulate, connected by a narrow isthmus when mature, globose to subglobose, smooth, nearly hyaline or pale salmon in mass, 8–11μ in diameter, mostly 8–9μ (pl. 2, D).

Perithecial stage: Perithecia gregarious or scattered, superficial or immersed when grown on agar media, smooth or loosely and sparsely soft hairy when superficial, yellowish when young, becoming dark brown to black; 250–300μ in diameter; wall medium thick, subcoriacious; ostiole obtuse, papillate; paraphyses (?) crowded, septate, collapsing and disappearing as soon as the asci appear; ascospores uniseriate or somewhat overlapping, oblong-elliptical with about 20 longitudinal, sometimes branched ridges, olivaceous at first, becoming dark brown to black, 29–35$\times$14–16μ, mostly 30–31$\times$15μ (pl. 4, C; pl. 3, C). This species is homothallic.

Habitat: Substratum unknown, Surinam, Dutch Guiana, Stahel.

Type: Culture No. 5578, slide No. 5579 from which plate 4, C, was taken. In mycological collections, Bureau of Plant Industry.

On bread the conidia are formed in abundance and have the same general character and appearance as *M. sitophila*.

The cultures of this came originally from Gerald Stahel, Government mycologist, Parimaribo, Dutch Guiana, and were sent the writers from the Central bureau voor Schimmelcultures at Baarn, Holland. These cultures when received contained perithecia.

Neurospora erythraea (Möll.) comb. nov.

Conidial stage (*Monilia erythraea* comb. nov.): Tufts abundant bright orange color in mass; fertile hyphae dichotomously branched, conidia catenulate globose to subglobose, smooth, 7 to 11μ diameter, mostly 8 to 9μ (pl. 2, A).

Perithecial stage: Perithecia gregarious, superficial subglobose loosely and sparsely soft hairy, yellow at first becoming reddish and finally dark brown, thick walled, subcoriacious, 400 to 600μ diameter; ostiole obtuse, short conical; asci cylindrical, stipitate, gelatinous ring at apex, 4-spored, about 250μ long; paraphyses lacking in mature perithecia; ascospores overlapping uniseriate, elliptical with 20 to 22μ longitudinal somewhat branched ridges, yellowish at first, becoming greenish black to black, 28–38$\times$13–18μ, mostly 34–36$\times$15–16μ. This species is apparently homothallic (pl. 4, D).

Habitat: On corn bread and burned stumps of "Rocas" Blumenau, Brazil, A. Möller (*34, p. 294*).

Type: No. 190 (5580). Slide No. 5581. From Möller's collection in alcohol in Botanical Museum, Berlin. In mycological collections, Bureau of Plant Industry.

The above description and Plate 2, D, were made from a part of Möller's original culture sent by H. W. Wollenweber of Berlin, which shows only the perithecia with 4-spored asci. Möller also mentioned finding perithecia with 8-spored asci associated with similar conidia. The 8-spored form was found on a burned stump of orange and was thought by him to be merely a form of *Neurospora erythraea*. It is more probable, however, that this was a distinct species.

Having no living material of Möller's plant, the writers can not prove that it was homothallic. The fact that he obtained perithecia in cultures made by the transfer of a bit of hyphae and that the writers' 4-spored species is homothallic would indicate the probability that such is the case in *Neurospora erythraea*. This species is distinguished from *N. tetrasperma* by its much larger perithecia and ascospores.

HETEROTHALLISM IN THE ASCOMYCETES

The discovery of heterothallism in the Mucorineae by Blakeslee (*4*) and the work on heterothallism in the higher basidiomycetes inaugurated by Mlle. Bensaude (*1*) and Kniep (*26*) need not be reviewed

here. Proof that species of ascomycetes are also heterothallic has been produced in but few cases. The work of Edgerton (*14*) has been frequently cited as showing that *Glomerella cingulata* is heterothallic. Edgerton makes no such claim in his paper. He distinctly states that both the strains produced perithecia when grown alone. However, when the same two strains were grown together on opposite sides of a Petri dish culture more perithecia were produced along the line where the mycelia met than when the strains were grown separately. This is a well known phenomenon. Many instances have been cited in the literature showing that bacteria or other fungous contaminations as well as different colonies of the same fungus often have a beneficial effect in changing the constitution of the medium or in otherwise stimulating the production of ascocarps. The erroneous idea seems to prevail that when fruiting bodies are produced along a line where two mycelia meet this is an indication of heterothallism.

Ascobolus magnificus was the first ascomycete to be definitely proved by the combination of monosporous cultures to be heterothallic (*13*). Only certain pairs of such cultures gave rise to ascocarps. Monosporous mycelia always remained sterile. This species produces functional ascogonia and antheridia which appear to rise from different hyphae. No sex organs are produced in monosporous cultures. In all probability the gametophytes come to maturity and produce their reproductive structures only when grown together in the same medium, the mycelium of each sex so changing the nature of the medium as to stimulate the development of reproductive structures on the mycelium of the opposite sex.

Kirby (*23*) has reported that *Ophiobolus graminis* is heterothallic, ascocarps being produced only when cultures are grown together in certain combinations, monosporous cultures remaining sterile. Kirby believes that the fertile strains are sexually differentiated. Davis (*8*), working with monosporous cultures of the same fungus originally isolated by Kirby, finds that ascocarps are developed in abundance from monosporous cultures. He has repeatedly isolated other single spore cultures of the same species which likewise developed ascocarps. Davis finds that strains from Arkansas and Kansas could not be induced to sporulate. Kirby in recent correspondence with the writers as a result of further work has modified his published statements regarding the heterothallism of this fungus. In view of the fact that Davis states that this Ophiobolus is homothallic and not heterothallic as claimed by Kirby, it is clear that further study on this species is necessary before the question can be considered settled.

Derx (*11*) working with species of Penicillium has proved conclusively that *Penicillium luteum* is heterothallic. Several single ascospore cultures were isolated and grown in all possible combinations. He found that about half the ascospores were of one sex and half the other.

Betts (*3*) has proved beyond a doubt that *Ascobolus carbonarius* is also heterothallic. He grew cultures from seven spores from a single ascus and found that three of them belonged to one sex and four to the other.

CULTURE STUDIES

The material used for cultures has been obtained from different countries, as noted in part in connection with the discussion of the three species of Neurospora described above. This material turned out to be of two different kinds. In all cases each culture had developed the Monilia stage more or less luxuriantly at the time it was received. A few of the cultures had developed ascocarps in transit; one culture produced perithecia later when transferred to a more suitable medium. Each collection was at first designated either by a name, number, or source without particular regard to its specific identity, which was in most cases not determined until later, as will be explained in connection with Table 6. It may be said here that with the exception of Nos. 1, 2, and 15, all of these cultures were later referred to *Neurospora sitophila*. A list of the cultures used, together with their history as far as known to the writers at the time this work was done, follows:

1. *Neurospora crassa*. The material for the work with this form was obtained from Charles Thom. It has been made the type of *N. crassa*. It was originally obtained in 1924 from sugar-cane bagasse near New Orleans.
2. S 227. A culture sent in by J. E. Flynn, Cornell University, who obtained it from H. H. Whetzel.
3. Herter. This material was obtained from the Centraalbureau voor Schimmelcultures at Baarn, Holland. It came originally from Herter and is presumed to be the same material studied by him. It has been made the type of *Neurospora sitophila*. The culture developed mature ascocarps and an abundance of conidia.
4. Japanese. The culture containing ascocarps and conidia received from Doctor Kitasima, Tokyo, Japan. The culture was labeled "single ascospore."
5. Arlington. The culture of the fungus which appeared as a contamination in Petri dish cultures in the greenhouse at Arlington Experiment Farm, Rosslyn, Va. After transfers to corn-meal agar in test tubes this material developed ascocarps as well as conidia.
6. B. P. I. A culture obtained from a contamination in a Florence flask containing fruit juice in a laboratory at the Department of Agriculture, Washington, D. C.; appeared about the same time as No. 5, the Arlington culture, and may have been originally from the same source. Ascocarps and conidia developed on the fruit juice in the flask.
7. Scales. A culture originally sent by F. M. Scales, of Mount Vernon, N. Y., obtained by him from a bakery in New York City. The culture as originally received produced a great abundance of conidia. A large number of subcultures obtained by transfer from the original material to corn-meal agar developed ascocarps.
8. Bainier. A culture obtained from Thom, as representing the Bainier material kept in culture in l'Ecole de Pharmacie, at Paris. This culture developed conidia and numbers of small sclerotiumlike bodies, but no ascocarps.
9. B 704. Culture obtained from the laboratory of H. H. Whetzel, who had originally received it from Wooster, Ohio, produced only conidia.
10. Rose. A culture which developed only conidia. Contributed by D. H. Rose, who had obtained the fungus from rotting strawberries at Chadbourn, N. C.
11. Columbia University. A culture from Columbia University. According to the history of the culture it was originally obtained from a bakery in St. Louis, Mo., by Kunkel (*28*).
12. Berkhout. A culture which developed conidia and numbers of very small bulbils or sclerotia was a part of the material reported on by Christine Berkhout (*2*).
13. Kauffman. Material contributed by C. H. Kauffman, Ann Arbor, Mich., produced only conidia.
14. Cooley. A culture obtained by J. S. Cooley from an apple-receiving room in a cold-storage plant at Charlottesville, Va. The same fungus had been obtained repeatedly from the air in apple-packing sheds.
15. *Neurospora tetrasperma*. A culture obtained from the Centralbureau voor Schimmelcultures at Baarn, Holland. The culture was originally contributed

by G. Stahel from Surinam. The culture had developed large numbers of ascocarps when received and only a very few conidia. Like *N. erythraea*, the species described by Möller (*34, p. 294*), each ascus develops only four spores.

SPORE GERMINATION

The conidia of the Monilia stage germinate readily under all ordinary cultural conditions. According to the statements of Tokugawa and Emoto (*48*) and others, the conidia when in a fairly dry condition resist for 15 or 20 minutes temperatures considerably above the boiling point of water.

The writers have found that in case of the *Monilia sitophila* there are a number of factors that must be considered in determining the effect of heat on germination. When the conidia are thoroughly wet most of them are killed when heated for five minutes at 65° C. and all are killed when kept at 72° C. for the same time. Should a little air be caught in a tangle of conidia and fragments of hyphae, some of the conidia will not be wet even though immersed, and these will survive when heated at much higher temperatures. It has also been found that, as reported by Thom and Ayers (*46*), heating of the conidia delays their germination sometimes for several days, so that the observations should be made for a period of at least 10 days after the experiment has been made.

In this connection Thom and Ayers in studying the effect of Pasteurization on mold spores found that some of the conidia of *Aspergillus fumigatus*, for example, germinated after having been floated on the surface of milk in a test tube and heated for 30 minutes at 65.6°C. These authors found that nearly all of the conidia of the different molds tested were killed when immersed in milk at 68.3° for 30 seconds.

The question as to whether or not conidia in flour survive the baking process would depend on whether they are thoroughly wet in mixing the dough, how porous the dough is after rising, and whether they start to germinate before being heated. The size of the loaf, the temperature of the oven, the length of time the loaf is baked, and the rapidity with which the loaf is cooled are some of the factors that must be taken into account.

Another important factor not heretofore considered is the presence of ascospores of the fungus. As will be pointed out later, the ascospores are much more resistant to heating under moist conditions than are the conidia. Experiments are being carried out in cooperation with L. H. Bailey of the Bureau of Chemistry to determine more definitely the effect of various temperatures and conditions of baking upon the viability of spores contained in the dough.

For the study of heterothallism the conidia alone could not serve as the primary source of pedigreed cultures. The ascospores rarely germinate under ordinary culture conditions. Möller (*34, p. 294*) was unable to obtain germination of ascospores of *Neurospora erythraea*. Kitasima (*25*) evidently had great difficulty in securing cultures from ascospores. Any attempt to obtain pedigreed cultures from spores which so rarely germinate under ordinary conditions would be a very tedious process and would often result in contaminations by conidia of the species. In order to secure numerous ascospore cultures it was necessary to find some method of insuring germination of the ascospores.

The method of germinating ascospores of certain species of Ascobolus described by Dodge (*12*) and further tested out by Ramlow (*39*) and Betts (*3*) was tried out and found to be highly satisfactory. The ascospores are sown on the surface of an agar medium in a Petri dish and spread out so that the spores may be picked out separately later. The Petri dish is then set in any ordinary drying oven where the temperature can be raised gradually from room temperature to 90° C. The thermostat may be so regulated as to require 20 or 30 minutes to do this. The exact temperature to which the spores are raised is not of vital importance. It will be found that a large percentage of ascospores so treated will begin to germinate within four or five hours. Transfers of single germinating spores must be made within a few hours, since the germ tubes grow rapidly and tend to become interlaced with those from other spores. A spore usually germinates from a pore in each end. A rather large globule is first extruded and from this vesicle usually two germ tubes develop and grow out in opposite directions (pl. 3, A, B, C).

When quantities of conidia are carried over with the ascospores in making the sowings, it is advisable to allow the cultures to stand a few hours so that the conidia may begin to germinate. The heating necessary to stimulate ascospore germination will kill the germinating conidia and facilitate the isolation of single ascospores. A better way is to use only material in which the perithecia are beginning to discharge the spores, which are shot some distance. The bottom of a Petri dish containing agar may be inverted over the culture bearing the perithecia so as to catch the ascospores as they are shot from the fruit bodies. In this way very few conidia will be carried up to the agar and the ascospores will be scattered far enough apart on the surface of the agar to be removed separately with little difficulty after germination. The percentage of germination is ordinarily high when the spores have been properly heated. The higher the temperature within the limits given above the more satisfactory will be the results of attempts to obtain cultures free from conidial contamination.

It has been found that corn-meal agar is an excellent medium for this culture work. Conidia are not produced in such abundance on this medium as on some others. While the three species studied produce perithecia under proper conditions when grown on corn-meal agar in Petri dishes, it is much better to use tube cultures. It goes without saying that in working with a fungus which produces conidia in such masses certain precautions are necessary to avoid contaminations, especially when material from many different sources is to be handled.

NEUROSPORA CRASSA

Following the culture methods described above, over 100 single ascospore cultures have been obtained. These were supplemented by several single conidium cultures, and mixed cultures derived from several ascospores were also made for comparison.

Svedelius (*45*) and others are now using the term "haplont" to distinguish a particular plant of the gametophytic phase of an alga or fungus. In the case of such a fungus as Neurospora, the gametophytic growth originating from a single ascospore is capable of

reproducing itself indefinitely by fragments of the hyphae or by the formation of conidia. The mycelium or haplont derived in this way would be analogous merely, but not homologous, to a clon in the higher plants. While no one is now misled by such statements as growing, pairing, or mating cultures or strains in different combinations, it is simpler in this connection to refer to monosporous cultures as haplonts.

The monosporous mycelia (haplonts) at first produced only conidia. After about 10 days some of them began to form structures which in size and color look like young perithecia. Such sclerotium-like bodies resembling the bulbils of certain species of the form genus Papulospora have been noted by several persons who have worked with *Monilia sitophila*, as already mentioned. These bodies were observed for several months but none developed ascospores. Several hundred subcultures were made by transferring the tips of hyphae or by transferring conidia; no perithecia were produced in these cultures. The mixed cultures, however, which were derived from three or four ascospores, usually developed perithecia within two weeks.

The cultures originally derived from single ascospores have been grown in different combinations. The results obtained by pairing 15 different haplonts in all possible ways, and in duplicate, are tabulated so that the cultures fall into two groups. (See Table 1.) The marginal and top numbers are used to designate particular cultures. Failure to produce perithecia is indicated by the minus sign; a positive sexual reaction and the formation of normal perithecia is indicated by the plus sign. The table shows that any mycelium in one group when grown with any other mycelium of the same group produces only conidia; when a mycelium from one group is grown with any mycelium from the other group perfect perithecia as well as conidia are matured. The results of this work prove conclusively that *Neurospora crassa* is heterothallic.

TABLE 1.—*Monosporous mycelia or haplonts, from random selection of ascospores of Neurospora crassa; haplonts paired in all possible ways and in duplicate* [a]

	1	4	5	8	16	19	2	3	6	7	9	10	11	12	20
1	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+
4	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+
5	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+
8	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+
16	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+
19	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
3	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
6	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
7	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
9	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
10	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
11	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
12	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
20	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−

[a] Marginal and top numbers refer to particular cultures. Failure to produce perithecia indicated by −. A positive sexual reaction and the formation of normal perithecia is indicated by +.

Each ascus of this species contains eight spores which are shot violently from the ascus at maturity. Occasionally the eight spores so discharged fall in a separate group. They can then be removed separately and germinated. Five sets of such haplonts, each set

consisting of eight monosporous mycelia derived from spores from the same ascus, were made. The haplonts from each set were then grown in all possible combinations. The results obtained with each of the five sets agree. Table 2 shows the results of mating the mycelia in one set. The marginal and top numbers represent the haplonts from single spores picked up at random after they had been discharged from a single ascus. The results show clearly that four spores from an ascus belong to one sex and four to the other.

TABLE 2.—*Eight monosporous mycelia or haplonts of Neurospora crassa. Spores from the same ascus, picked up at random after their discharge. All combinations* [a]

	1	2	6	7	3	4	5	8
1	−	−	−	−	+	+	+	+
2	−	−	−	−	+	+	+	+
6	−	−	−	−	+	+	+	+
7	−	−	−	−	+	+	+	+
3	+	+	+	+	−	−	−	−
4	+	+	+	+	−	−	−	−
5	+	+	+	+	−	−	−	−
8	+	+	+	+	−	−	−	−

[a] Marginal and top numbers refer to particular cultures. Failure to produce perithecia indicated by −. A positive sexual reaction and the formation of normal perithecia is indicated by +.

NEUROSPORA SITOPHILA

Ascocarpic material of *Neurospora sitophila*, as typified by the Herter material referred to above, has been received from five different sources. The first and most extensive culture work with this species was done with material collected at Arlington, Va. Ninety-six cultures were obtained by germinating single ascospores. These mycelia were grown in several thousand cultures on different media, singly and in different combinations. Monosporous mycelia produce only conidia and in most cases numerous sclerotiumlike bodies. The 96 mycelia were not grown in all possible combinations, but it was proved conclusively that the Arlington material is heterothallic. The results obtained by later cultures of the Herter and Japanese material, which are the only results presented here in tabulated form, substantiate those previously obtained.

A number of haplonts derived from single ascospores of the Herter material have been grown for several months, both alone and in all possible combinations. The results obtained by growing in all possible combinations eight of such mycelia chosen at random are shown in Table 3. The cultures used may be arranged in two groups. Perithecia are produced only when a mycelium from one of the groups is grown together with a mycelium from the other group. For example, mycelium 4 develops only conidia when grown alone or in combination with either mycelium 2 or 6. When, however, haplont 4 is grown with either mycelium 1, 3, 5, or 8, both conidia and perithecia are developed. The Herter material of *Neurospora sitophila* is also heterothallic.

The culture of the Japanese material of *Neurospora sitophila* was maturing perithecia when received. Twenty haplonts or monosporous cultures from ascospores were obtained and subcultures from these

have been grown for nearly a year. In no case has such a culture produced perithecia. Eleven of these haplonts were grown in combination, as shown in Table 3. This shows that the Japanese material of *N. sitophila* is likewise heterothallic. The haplonts fall into two groups. Any mycelium from one group will produce perithecia when grown with a mycelium from the other group. Even though all the cultures of one group be grown together no perithecia are formed.

TABLE 3.—*Monosporous mycelia (haplonts) obtained by random selection of ascospores of Neurospora sitophila (from Herter). All combinations* [a]

	2	4	6	1	3	5	7	8
2	−	−	−	+	+	+	+	+
4	−	−	−	+	+	+	+	+
6	−	−	−	+	+	+	+	+
1	+	+	+	−	−	−	−	−
3	+	+	+	−	−	−	−	−
5	+	+	+	−	−	−	−	−
7	+	+	+	−	−	−	−	−
8	+	+	+	−	−	−	−	−

[a] Marginal and top numbers refer to particular cultures. Failure to produce perithecia indicated by −. A positive sexual reaction and the formation of normal perithecia is indicated by +.

The original culture from Doctor Kitasima was according to the label, obtained from a single ascospore. Without denying that a culture from a single ascospore of this material might for some very definite reason develop ascocarps, the writers merely wish to state here that all of their single-spore cultures from this material produce only conidia and sclerotiumlike bodies when grown alone. Table 4 shows the results of mating haplonts from these cultures.

It is unnecessary to describe in detail the results obtained by growing large numbers of monosporous cultures of this species from ascosporic material received from the other three sources mentioned. The results prove beyond question that the species *Neurospora sitophila* is heterothallic and that single-spore cultures so far obtained have not produced perithecia.

As stated above, the original material from each of the five sources contained both of the elements necessary for sexual reproduction of the species. Frequently, however, mycologists find that cultures of *Monilia sitophila* obtained from rotting fruit, from bakeries, or from burned trees produce only conidia, regardless of what culture medium is employed. A number of such cultures of the Monilia stage have been received within the present year. In some cases hundreds of subcultures were grown in attempts to obtain ascocarps, but without success. It became a matter of much interest to determine whether perithecia could be obtained by mating two or more of these forms together in a culture. The results of this work, discussed further in connection with Table 6, show that cultures may be classed in two groups, based on their sexual reactions. All but one of the conidial cultures received may be referred to the species *Neurospora sitophila* or a species so similar to it morphologically as to be indistinguishable from it.

TABLE 4.—*Monosporous cultures, obtained by random selection of ascospores of Neurospora sitophila (Japanese). All combinations* •

	1	3	5	8	9	12	2	4	6	10	11
1	−	−	−	−	−	−	+	+	+	+	+
3	−	−	−	−	−	−	+	+	+	+	+
5	−	−	−	−	−	−	+	+	+	+	+
8	−	−	−	−	−	−	+	+	+	+	+
9	−	−	−	−	−	−	+	+	+	+	+
12	−	−	−	−	−	−	+	+	+	+	+
2	+	+	+	+	+	+	−	−	−	−	−
4	+	+	+	+	+	+	−	−	−	−	−
6	+	+	+	+	+	+	−	−	−	−	−
10	+	+	+	+	+	+	−	−	−	−	−
11	+	+	+	+	+	+	−	−	−	−	−

• Marginal and top numbers refer to particular cultures. Failure to produce perithecia indicated by −. A positive sexual reaction and the formation of normal perithecia is indicated by +.

It may be of interest to know, for example, that *Monilia sitophila* obtained from rotting strawberries at Chadbourn, N. C., forms perithecia when grown together with the *M. sitophila* with which Berkhout (*2*) worked in Holland. Both collections produce only conidia when grown separately. The culture from a St. Louis bakery (*28*) and the one from the Bainier collection do not develop perithecia when grown separately or together, but each develops perithecia when paired either with the *M. sitophila* received from a laboratory at Ann Harbor, Mich., or with a culture sent in by Bunzel from a bakery in New York City.

NEUROSPORA TETRASPERMA

As already stated, the culture originally obtained from Doctor Stahel from Surinam had matured large numbers of perithecia when received. The species is characterized especially by the fact that the asci, with few exceptions, contain only four spores. The species produces very few conidia in culture on corn-meal agar, but when grown on bread or such a medium as potato-dextrose agar great quantitites of conidia are formed, so that in the conidial stage it would not be readily distinguished from the red-mold stage of *Neurospora sitophila*. Growing the two species side by side on corn-meal agar, however, would enable one to distinguish readily the Surinam species, which has been described above under the name *N. tetrasperma*.

A number of monosporous cultures were obtained by isolating single ascospores from the heavy spore print formed on the tube of the original culture. Within six or seven days each of these cultures showed mature ascocarps which were discharging spores. This proved conculsively that *Neurospora tetrasperma* is commonly homothallic.

Over 200 additional monosporous mycelia obtained from other ascosporic subcultures of this species have developed perithecia on corn-meal agar.

Möller (*34, p. 294*) noted that in *Neurospora erythraea* the ascospores varied considerably in size. The average size, he stated, was about 36μ long. As observed previously under the discussion of this species, it has been found that the ascospores of the Surinam material, *N. tetrasperma*, average about 30 to 32μ long. These measurements apply to spores from an ascus bearing 4 spores. Careful examinations

of crushed preparations show that occasionally an ascus develops 5 or 6 spores, but more rarely 3, 2, or only 1 spore. In case an ascus develops 5 spores 2 of them will be very small, while the other 3 will be of normal size. An ascus with three spores ordinarily will have 1 spore which is about 40 to 45μ long. One-spored asci are very rare. In the 2 or 3 that have been found the spores were from 60 to 65μ long. The smallest spores are about 20 to 25μ long. Over 50 monosporous cultures of the abnormally large spores have been made. One has no difficulty in distinguishing these large spores from the common type. With the exception of two somewhat doubtful cases which need further study, the results show that each of the unusually large spores possesses all the elements necesary for sexual reproduction and produces good perithecia.

In the case of the very small spores it has been found that the size varies considerably so that it is not always possible to distinguish these small spores definitely from the others. Forty-four monosporous cultures from spores which were judged to be under size were isolated. Only five of these cultures developed perithecia. The others developed large numbers of very small sclerotiumlike bodies and a few conidia. The cultures that remained sterile were then grown in all possible combinations. The results of growing 21 of these haplonts together is shown in Table 5. It will be seen that the 21 cultures fall into two groups. There are 9 cultures in one group and 12 in the other. Mycelium from one group produces perithecia only when grown in combination with mycelium from the second group. In other words, this species which is ordinarily homothallic occasionally develops spores which are not totipotent. Each spore contains only one of the sex elements. By selecting at random the small spores, haplonts may be obtained which when grown in combination develop normal perithecia with asci which produce predominantly only four spores. The results of all the culture work with this species show that regardless of whether perithecia are obtained by growing what may be called the normal spores, or by growing those that are especially large or very small, the resulting perithecia and ascospores are of the same type.

TABLE 5.—*Small spore selections of Neurospora tetrasperma (commonly homothallic) which are heterothallic* [a]

	1	3	D	8	30	32	E	H	J	2	4	6	7	10	28	29	31	33	34	G	5
1	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
3	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
D	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
8	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
30	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
32	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
E	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
H	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
J	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
4	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
6	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
7	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
10	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
28	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
29	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
31	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
33	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
34	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
G	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
5	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−

[a] Marginal and top numbers refer to particular cultures. Failure to produce perithecia indicated by −. A positive sexual reaction and the formation of normal perithecia is indicated by +.

IDENTIFICATION OF CULTURES BEARING ONLY CONIDIA

Certain cultures which developed only conidia of the Monilia stage are listed on page 1029. In their conidial stage the two heterothallic species are not readily distinguished with certainty. The most practical way by which the species affiliations of the sterile cultures could be determined was to grow them in combination with monosporous cultures of known origin. If, for example, a culture of unknown identity be mated with each of two reciprocal haplonts of *Neurospora crassa*, and if in one case perithecia are produced similar to those normally developed by *N. crassa*, such a result would indicate that the unknown culture probably belongs to that species. In Table 6 the reciprocal haplonts of a species or those which when paired will produce perithecia are designated for convenience as A and B without regard to their sex, which is unknown at present. The unknown strains were grown in combination with several different pairs of reciprocal haplonts of each of the two species *N. crassa* and *N. sitophila*. The results shown in Table 6, as previously stated, prove that with one exception each unknown was one or the other sex of *N. sitophila*.

PERITHECIA RESULTING FROM MATING HAPLONTS OF DIFFERENT SPECIES

For purposes of experiments on crossing or hybridization one may consider as heterothallic the small spore selections of the homothallic species, *Neurospora tetrasperma*, discussed in connection with Table 5. Pairs of reciprocal haplonts of each of the three species and all of the sterile cultures of unknown identity noted above were grown in all possible combinations. These results are also included in Table 6. In case no perithecia were produced the result is indicated as before with the minus sign. When the haplonts mate and perithecia result from the combination this is indicated by the plus sign, and the strains crossed are assumed to belong to the species having a perithecium and spores of the same kind. The results of crossing reciprocal strains or haplonts of one species with reciprocal strains of another species are indicated by the minus sign when no sexual reaction occurred, and by the letter X when the reaction results in the formation of ascocarps with mature spores. In these cases the course of development of the perithecia and ascospores differs from that of either species. There are various differences in the form and size of the spores, and the lack of uniformity in reaching maturity is such as would be expected in hybrids.

TABLE 6.—*Haplonts of unknown identity and those known to belong to Neurospora sitophila (from various sources), N. crassa (Thom coll.), and N. tetrasperma (Stahel coll.), paired in all possible combinations* [a]

	N. sitophila from—					Unknowns from—							N. sitophila from—					Unknowns from—						
	Herter A	Japanese A	Arlington A	B. P. I. A	Scales A	Bainier	B 704	Rose	Columbia University	N. crassa A	N. tetrasperma from—tetrasperma A		Herter B	Japanese B	Arlington B	B. P. I. B	Scales B	Berkhout	Kauffman	Cooley	S 227	N. crassa B	N. tetrasperma from—tetrasperma B
N. sitophila:																							
Herter A	–	–	–	–	–	–	–	–	–	–	–		+	+	+	+	+	+	+	+	X	X	X
Japanese A	–	–	–	–	–	–	–	–	–	–	–		+	+	+	+	+	+	+	+	X	X	X
Arlington A	–	–	–	–	–	–	–	–	–	–	–		+	+	+	+	+	+	+	+	X	X	X
B. P. I. A	–	–	–	–	–	–	–	–	–	–	–		+	+	+	+	+	+	+	+	X	X	X
Scales A	–	–	–	–	–	–	–	–	–	–	–		+	+	+	+	+	+	+	+	X	X	X
Unknowns:																							
Bainier	–	–	–	–	–	–	–	–	–	–	–		+	+	+	+	+	+	+	+	X	X	X
B 704	–	–	–	–	–	–	–	–	–	–	–		+	+	+	+	+	+	+	+	X	X	X
Rose	–	–	–	–	–	–	–	–	–	–	–		+	+	+	+	+	+	+	+	X	X	X
Columbia University	–	–	–	–	–	–	–	–	–	–	–		+	+	+	+	+	+	+	+	X	X	X
N. crassa A	–	–	–	–	–	–	–	–	–	–	–		X	X	X	X	X	X	X	+	+	+	X
N. tetrasperma A	–	–	–	–	–	–	–	–	–	–	–		X	X	X	X	X	X	X	X	X	X	+
N. sitophila:																							
Herter B	+	+	+	+	+	+	+	+	+	X	X		–	–	–	–	–	–	–	–	–	–	–
Japanese B	+	+	+	+	+	+	+	+	+	X	X		–	–	–	–	–	–	–	–	–	–	–
Arlington B	+	+	+	+	+	+	+	+	+	X	X		–	–	–	–	–	–	–	–	–	–	–
B. P. I. B	+	+	+	+	+	+	+	+	+	X	X		–	–	–	–	–	–	–	–	–	–	–
Scales B	+	+	+	+	+	+	+	+	+	X	X		–	–	–	–	–	–	–	–	–	–	–
Unknowns:																							
Berkhout	+	+	+	+	+	+	+	+	+	X	X		–	–	–	–	–	–	–	–	–	–	–
Kauffman	+	+	+	+	+	+	+	+	+	X	X		–	–	–	–	–	–	–	–	–	–	–
Cooley	+	+	+	+	+	+	+	+	+	X	X		–	–	–	–	–	–	–	–	–	–	–
S 227	X	X	X	X	X	X	X	X	X	+	X		–	–	–	–	–	–	–	–	–	–	–
N. crassa B	X	X	X	X	X	X	X	X	X	+	X		–	–	–	–	–	–	–	–	–	–	–
N. tetrasperma B	X	X	X	X	X	X	X	X	X	+	X		–	–	–	–	–	–	–	–	–	–	–

[a] In this table A and B are reciprocal as to sex reaction. The minus sign indicates that no perithecia were produced; the plus sign indicates that normal perithecia developed; the letter X indicates that hybrid perithecia with mature asci were formed.

As noted above the table shows that with the exception of S 227 all of the cultures received which were sterile with regard to ascocarps when grown alone, belong to the species *Neurospora sitophila*. The culture S 227 formed perfect perithecia when grown with haplont A of *N. crassa*, so that it is known to belong to the species *N. crassa*, whose perithecium has the same characters. After these results were obtained and the history of the culture further determined it was found that Whetzel originally received the culture from Thom, who had also contributed the material for the culture work conducted by the writers on *N. crassa*. In making the transfers from Thom's cultures which were producing perithecia, only the B strain was sent to Whetzel. At least the culture S 227 with which the writers did their work remained sterile as long as it was cultured alone.

Any haplont A of the species *Neurospora crassa* and a haplont B of *N. sitophila* when mated develop a few large perithecia which

mature a relatively small number of ascospores. A positive reaction occurs when the heterothallic haplonts A of *N. tetrasperma* are mated with haplonts B of either *N. crassa* or *N. sitophila*, resulting in the development of perithecia with ascopores.

At this writing no culture work has been done with spores from perithecia which may be true sexual hybrids between the species; that is, the ascocarps which are developed when strains of different species are crossed may readily be produced as the result of unions between reciprocal haplonts. Further work will be necessary before any conclusion can be reached concerning the nature of these structures. The results of cytological work on Neurospora will be published in another connection.

SUMMARY

The red bread-mold fungus, *Monilia sitophila* Mont., was first described in 1843. It has frequently been found seriously infesting bakeries in Europe and America. It has also been reported on various fruits and tree trunks and stumps recently killed by burning, and on sugar-cane bagasse.

This study shows that instead of one species there are at least four which have been passing under this name. The life histories of these four species have been determined.

Instead of belonging to Sclerotinia, as might be expected from the great resemblance of the conidia to those of *Monilia cinerea*, the ascospore stage is found to be a pyrenomycete which seems to belong to an undescribed genus, which is named Neurospora.

The conidia of three species grow readily at ordinary laboratory temperatures. When exposed to a temperature of 120° C. for 30 minutes dry conidia are killed; when wet they are killed when exposed to a temperature of 72° for 5 minutes. The ascospores are more resistant when dry.

The ascospores do not germinate under ordinary cultural conditions, but grow readily after heating in a moist condition at 65° to 70° C. for a few minutes.

Baking tests show that 1-pound loaves in which have been placed both conidia and ascospores and which have been baked in an oven for 35 minutes at 215° C. do not develop mold.

Bakeries contaminated with these fungi can be freed from them by thorough cleaning of walls, floors, and utensils with live steam, soap and water, formaldehyde, or any good antiseptic.

Two of the species, *Neurospora sitophila* and *N. crassa*, have been found to be heterothallic. *N. tetrasperma* is usually homothallic, but occasionally heterothallic haplonts have been obtained by the selection of abnormal spores. *N. erythraea* has not been studied in culture by the writers, but is probably homothallic. Cultures from single spores of the heterothallic species produce only conidia. Reciprocal haplonts when mated produce perithecia.

Crossing the different species by mating their haplonts results in the formation of perithecia with ascospores which seem to have hybrid characters.

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A STUDY OF THE TOXICITY OF ACID LEAD ARSENATE ON THE JAPANESE BEETLE (POPILLIA JAPONICA NEWM.) [1]

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INTRODUCTION

During the past few years lead arsenate has been recommended for the control of the Japanese beetle. The results of its use show that this arsenical has given very satisfactory protection to the foliage, which may be attributed to the repellent action of acid lead arsenate on the beetle. The habits of the beetles are such that no accurate figures have been obtained on the percentage killed. The results of a large number of experiments, involving different methods, indicate that the kill is approximately 20 per cent of the beetles occurring on the sprayed foliage. These results, however, give no indication of the quantity of arsenic required to kill the beetle, or the quantity of sprayed foliage which it would have to eat to cause death. As a step to the solution of these problems the experiments reported in this paper were undertaken.

EXPERIMENTAL PROCEDURE

For several reasons it has been difficult to determine the value of a spray in the field. Of these, the following are important: (1) Many beetles which consume a lethal dose of arsenic probably leave the sprayed foliage before dying; (2) many beetles are repelled from sprayed foliage before they have taken a toxic dose; and (3) the beetles are incessantly active. For these reasons it seemed necessary to carry on the experiments in cages, so that the beetles might be kept under observation.

PREPARATION AND APPLICATION OF SPRAY

Suspensions of lead arsenate in water were prepared from samples of dry commercial lead arsenate containing 32 per cent, by weight, of arsenic oxide, as determined by analysis. Six mixtures of lead arsenate were used, varying progressively from 1 to 6 pounds of it to 50 gallons of water. To the mixture was added a little lime and casein mixture, to facilitate the spreading of the lead arsenate evenly over the surface of the foliage. Care was taken to use the smallest proportion of the lime and casein mixture consistent with satisfactory results, which proved to be 3.9 grams to 3 gallons of the mixture. To determine the various quantities of arsenic oxide (As_2O_5) remaining

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[2] The work here reported was done at the Japanese Beetle Laboratory during June, July, and August, 1923. The writer has had valuable assistance from H. S. Swingle on this project. Credit is also due L. B. Smith, in charge of the Japanese beetle investigations, for many suggestions given throughout the work, especially for the method used in determining the quantity of leaf area consumed by the individual beetles.

on the foliage, the leaves of several smartweed plants were first measured with a planimeter, and the plants were then dipped into one of the mixtures of lead arsenate and lime and casein mixture. As soon as the leaves were dry they were clipped off the plant and allowed to fall into a wide-mouthed bottle, which was then securely corked. The organic matter of the leaves was subsequently oxidized in the collecting bottle with sulphuric and nitric acids, and the arsenic oxide contained determined by a standard procedure. This method was used for each of the six mixtures and gave for each the total quantity of arsenic on leaves of known area. Table 1 shows the quantity of arsenic oxide per square inch of leaf surface, for the six mixtures of lead arsenate and lime and casein mixture used in the tests, and its equivalent expressed in terms of lead arsenate. The square inches, to a convenient fraction of a unit, could be read, leaf by leaf, from the planimeter. Since arsenic oxide constitutes 32 per cent, by weight, of the commercial arsenate used, the quantity of such arsenate equivalent to each determination of the oxide was readily found by multiplying by 3.125.

TABLE 1.—*Arsenic oxide, and its equivalent in lead arsenate, per unit area of leaves sprayed with mixtures of lead arsenate, lime and casein mixture, and water*

Quantity of lead arsenate per 50 gallons of water	Number of leaves measured and dipped	Leaf surface measured (both sides)	Quantity of arsenic oxide (As_2O_3) found in sample	Quantity of lead arsenate ($PbHAsO_4$) found in sample [a]	Arsenic oxide (As_2O_3) per square inch of leaf surface	Lead arsenate ($PbHAsO_4$) per square inch of leaf surface
Pounds		*Sq. in.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
1	32	204.46	1.6461	5.1441	0.0081	0.0252
2	35	195.90	4.5317	14.1616	.0231	.0723
3	34	209.16	9.0725	28.3516	.0434	.1355
4	54	340.94	16.5238	51.6369	.0485	.1515
5	57	375.26	19.5211	61.0034	.0520	.1626
6	35	181.88	10.0939	31.5434	.0555	.1734

[a] The lead arsenate is the chemical equivalent (in terms of the commercial lead arsenate used) of the arsenic oxide (As_2O_3) determined by analysis.

A comparison of the quantities of arsenic per unit area adhering to the smartweed foliage dipped into the mixtures containing various proportions of lead arsenate shows that the increase in arsenic is rather uniform to and including the third mixture. The difference in quantity of arsenic oxide adhering to the foliage in the case of that mixture and of the next is very slight. This similarity of effect may account for the results of the control spraying in the field, practically as good protection being obtained when the lead arsenate was used at the rate of 3 pounds to 50 gallons of water as when it was used at the rate of 4 pounds to the same quantity of water.

MEASUREMENT OF QUANTITY OF FOLIAGE EATEN

Potted smartweed plants, averaging 12 inches in height and free from injury, were dipped into the various suspensions of lead arsenate and lime and casein mixture. Great care was taken that all parts of a plant should be thoroughly wetted with the mixture. A small cage, 16 inches high and 6 inches in diameter, constructed of galvanized-wire gauze having 16 meshes to the inch, was placed over

each plant and a beetle introduced. The wire cage was firmly pressed down into the soil about the base of the plant, to prevent the escape of the beetle. These cages were kept in an outdoor open-type insectary, and several tests were made with them, an equal number of cages being used to each mixture, and four check cages. Equal numbers of male and of female beetles were used. No starvation tests were made, experiments having shown that beetles starved for 48 hours or more will feed readily upon poisoned foliage.

Hourly observations of the foliage eaten, the time of feeding, and the mortality of the beetles, were made only between the hours of 8 a. m. and 5 p. m. The greater part of the feeding was done during the period of observation, but some of it was carried on outside of this period. The fact that a number of beetles died while not under observation made it necessary to give the time in the tables as approximate, instead of as absolute. If a beetle was feeding at the time the observation was made, it was allowed to continue its feeding and a record was made of the fact. In many cases the beetle was in the soil at the time of observation; when this occurred, the beetle was dug up in order to determine whether it was living or dead. Many of the beetles were found dead in the soil about the base of the plant. The cages containing the poisoned foliage were kept in use until the observations were terminated by the death of the beetles, whereas the cages containing unsprayed foliage were discontinued as the beetles in them died, or as soon as all the experiments on poisoned foliage were ended. When a portion of a leaf had been eaten the leaf was clipped from the plant and a photographic print of it was made immediately, showing the actual size of the leaf and the area eaten. The area eaten and the background of the print were shown in black, and the remaining, uneaten area appeared white. In many cases where the area eaten was too small to be measured with a polar planimeter, it was enlarged 100 times in area by the use of a projectoscope. This apparatus projected the print on a screen, over which was traced the enlarged area. These areas could then be measured accurately by the polar planimeter and reduced by mathematical calculation to the corrected measurements.

CRITERIA OF TOXICITY

In order to judge the value of the methods described and the results obtained by using them, the following factors, impossible to control, are enumerated: (1) The beetles were gathered in the field at random, and varied in size and in age; (2) individual beetles vary as to quantities of arsenic necessary to kill; (3) some beetles die immediately after eating a lethal quantity of arsenic, whereas others are sick several days before dying; (4) the weather and the degree of hunger influence the time of feeding and the quantity consumed; (5) the distribution of arsenic on the foliage is somewhat uneven; and (6) the growth of the leaf during the experiment produces areas unprotected by arsenic. On account of these uncontrollable factors the results are valuable only for a rough comparison. In computing the toxic dose required to kill it was assumed that in all cases the arsenic oxide was evenly distributed over the foliage.

Table 2, based on several tests in each series, is a condensed summary of averages of the calculated quantity of arsenic required to kill, of the time from the beginning of the experiment to the death of the beetle, of the amount of leaf area eaten, and other important data. The results tabulated indicate how effective arsenic oxide may be as a toxic agent in the field, if its repellent action to the beetle can be overcome. The areas eaten were so small that for purposes of comparison it seemed better to express them in square millimeters rather than in the original units of measurement with the planimeter. The conversions were made by multiplying the areas in square inches by 645.1606; and in the case of each of the six mixtures of lead arsenate and lime and casein mixture, the quantity of arsenic oxide and of lead arsenate per beetle was found by multiplying the quantity per square inch of leaf surface by the area in square millimeters of leaf surface eaten, and dividing the product by 645.1606.

TABLE 2.—*Comparison of data obtained from the feeding of individual Japanese beetles on unsprayed smartweed foliage, with data obtained for beetles feeding on smartweed foliage sprayed with mixtures containing various proportions of lead arsenate*

Quantity of acid lead arsenate to 50 gallons of water	Number of beetles observed	Approximate average time from beginning of experiment to first feeding	Approximate average time from first feeding to death	Approximate average time from beginning of experiment to death	Average area of leaf surface eaten per beetle [1]	Average quantity of arsenic oxide eaten per beetle	Average quantity of lead arsenate eaten per beetle
		Hours	*Hours*	*Hours*	*Sq. mm.*	*Mgm.*	*Mgm.*
1 pound	23	28	54	82	90.44	0.0011	0.0035
2 pounds	34	21	67	88	73.26	.0026	.0082
3 pounds	31	20	66	86	74.20	.0050	.0156
4 pounds	33	23	48	71	63.32	.0048	.0149
5 pounds	31	20	64	84	38.46	.0031	.0097
6 pounds	17	26	42	68	48.10	.0041	.0129
Control (unsprayed)	33	22			[2] 2,405.16		

[1] The term "leaf surface" means the total surface of the upper and lower sides of the leaf. It does not mean the size of the hole made in the leaf.
[2] Not to be compared with sprayed foliage.

The data in Table 2 indicate that the area of leaf surface eaten per beetle tends to decrease as the arsenic content of the leaf surface increases. The average toxic dose, as shown by the table, varies from 0.0011 mgm. to 0.0050 mgm. of arsenic oxide (As_2O_5) and from 0.0035 mgm. to 0.0156 mgm. of lead arsenate ($PbHAsO_4$). The approximate average time from the beginning of the experiment to the first feeding varies from 20 hours to 28 hours. The approximate average time from the beginning of the experiment to the death of the beetle varies from 68 to 88 hours. The data indicate that a small percentage will die within 48 hours after the beginning of the experiment, and that only a small quantity of available arsenic will be needed to kill the beetle. Twenty-three beetles ate, before death occurred, an average of 90.44 sq. mm. of leaf area, sprayed with lead arsenate in the proportion of 1 pound to 50 gallons of water. Similarly, before dying, 34 beetles ate an average of 73.26 sq. mm. of leaf area, sprayed with lead arsenate at the rate of 2

pounds to 50 gallons of water; 31 ate an average of 74.20 sq. mm. of leaf area sprayed with 3 pounds to 50 gallons; 33 ate an average of 63.32 sq. mm. of leaf area sprayed with 4 pounds to 50 gallons; 31 ate an average of 38.46 sq. mm. of leaf area sprayed with 5 pounds to 50 gallons; and 17 ate an average of 48.10 sq. mm. of leaf area sprayed with 6 pounds of lead arsenate to 50 gallons of water. In the check cages, containing unsprayed foliage, 33 beetles ate an average of 2,405.16 sq. mm. of leaf area, only 3 beetles dying before the experiments were discontinued. However, the beetles in these cages fed for a longer time than did those in the cages containing the poisoned foliage.

SUMMARY

Various mixtures of lead arsenate were sprayed on smartweed and tested against 169 individual beetles. Observations were made as to the length of time of feeding, the area of foliage consumed, and the quantity of arsenic which caused death. The results indicate that small dosages of available arsenic are lethal, and that in case of ordinary infestations control in the field will be obtained without great injury by beetles to sprayed foliage. The protection afforded by a spray of lead arsenate, as shown by the small quantity of foliage eaten, is the outstanding fact established as a result of these experiments.

THE DEVELOPMENT OF THE PECAN NUT (HICORIA PECAN) FROM FLOWER TO MATURITY[1]

By J. G. WOODROOF, *Assistant in Horticulture*, and NAOMI CHAPMAN WOODROOF, *Assistant in Botany, Georgia Agricultural Experiment Station*[2]

INTRODUCTION

The increasing popular demand for information on the several regions or structures of the pecan nut (*Hicoria pecan* Brit.), their relative rate of growth, and the dates of the critical periods of their growth has indicated the need for a thorough study of the developing nut. The present paper is intended to supplement previous papers on morphological and physiological development of the buds and flowers of the pecan tree.

Stuckey (*5*)[3] studied 25 varieties of pecans during the flower stage and immediately following. His object was to study self-sterility in pecans and incidentally to note the morphological development.

The senior writer (*6*) studied the morphological development of the staminate flowers and briefly noted the development of the nut.

In a previously published article (*7*) the writers describe the time of fruit-bud differentiation and the subsequent development of the flowers. It was intended that such a study would lay an adequate foundation for a study of the nut itself.

Billings (*2*) studied fertilization in *Carya olivaeformis* Nutt. (*Hicoria pecan* Brit.). He found that the pollen tube passes down the axial tissue of the style to a point near the cavity of the ovary, where it turns and passes down the ovary wall close to the margin of the cavity. When a point is reached a little below the funiculus, the pollen tube curves toward the center, and when under the ovule turns upward to the embryo sac.

Nawaschin (*3*) described the method of fertilization in *Juglans regia*. He concluded that the pollen tube did not enter the micropyle but passed down the ovary wall and entered the ovule through the chalaza.

METHODS

Field notes and free-hand sections have been made annually for four years, and material has been collected for embedding in paraffin and sectioning with a microtome for two years. The first year embedding material was collected at 10-day intervals from the Alley and Frotscher varieties; the second year embedding material was collected every five days from the Alley and Nelson varieties. Material from the Stuart, Jerome, Alley, and Nelson varieties was taken twice each day for post-pollination studies. In the early stages the entire nut was embedded. As it grew larger only sections

[1] Received for publication Feb. 23, 1927; issued July, 1927. Published with the approval of the director as paper No. 22, Journal series, Georgia Agricultural Experiment Station.

[2] The writers wish to give credit to W. G. Friedemann, chemist, Georgia Experiment Station, for the analyses of pecan kernels; and acknowledge constructive criticisms from B. B. Higgins, botanist, and H. P. Stuckey, director of the station.

[3] Reference is made by number (italic) to "Literature cited," p. 1063.

through the nut were used, and when the nut was almost grown only certain regions were embedded.

The same 25 varieties used by Stuckey and Woodroof (*5*, *6*) were used for field notes and observation in this work.

Picric acid killing and fixing agent was found most satisfactory. Juel's fixative was satisfactory during the early stages but failed to fix the material well in later stages of growth. Carnoy's fixative caused shrinkage of the tissue and rendered it brittle. Flemming's weak fixative rendered the material too brittle to section well. Haidenhain's iron-alum haematoxylin was used for staining.

EXPERIMENTAL DATA

After pollination the surface of the stigmas become darkened and shrivel immediately. The entire stigmatic tissue, which extends to the region of the hull and shell, dies and becomes hard within two weeks after pollination. It is soon detached from the hull and shell regions and may drop off, or it may be held in place indefinitely by the vestiges of the bracts.

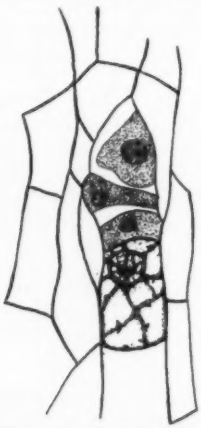

FIG. 1.—Four-megaspore stage, Stuart variety pecan, 18 hours after pollination. The three upper megaspores show indications of disintegration; the lower megaspore will develop into an embryo sac. × 830

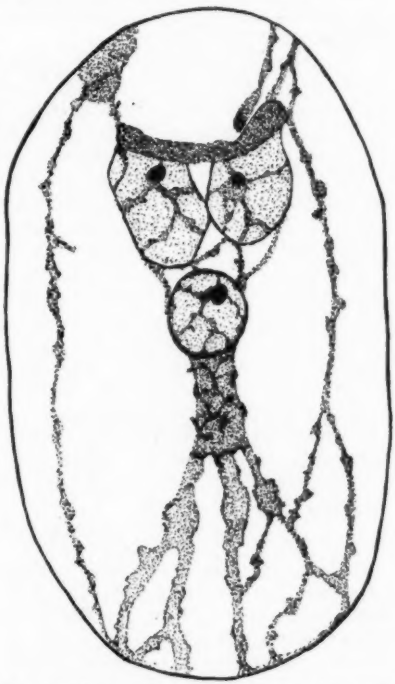

FIG. 2.—Mature egg apparatus, Alley variety pecan, June 7. × 830

To determine the course of the pollen tube has been found to be difficult. The largest number of germinating pollen grains appear on the outermost surfaces of the stigmas, and the early course of the pollen tube is inward toward the vascular bundles of the stigma. The pollen tube apparently follows this series of vascular bundles until it reaches the chalaza. The writers have not followed the course of the pollen tube in detail, but indications are that it reaches the embryo sac through the chalaza.

Beginning at pollination time, about April 26 to 31, the condition of the embryo sac, embryo, and pollen tube at subsequent dates are summarized in Table 1:

TABLE 1.—*Condition of embryo sac, embryo, and pollen tube at different periods, beginning at time of pollination*

Time	Condition of embryo sac and embryo	Condition of pollen and pollen tube
At pollination	4 megaspore stage	Pollen shed.
12 hours after pollination	3 megaspores disintegrating; lower megaspore developing (fig. 1).	Pollen germinating.
24 to 40 hours after pollination	3 megaspores disintegrating. Lower megaspore undergoes nuclear division.	Pollen tube growing.
2 to 5 weeks after pollination	Nuclear division in embryo sac; 8 nuclei formed. Antipodals disintegrate.	Do.
5 to 7 weeks after pollination	Mature egg apparatus. Probable fertilization with formation of embryo (fig. 2).	
9 weeks after pollination	Well-defined endosperm; 1 cell embryo, attached at top of cavity of embryo sac (fig 3).	
10 weeks after pollination	2 to 4 cell embryo	
11 weeks after pollination	Embryo 8 to 16 cells	
12 weeks after pollination	Embryo 32 to 64 cells	

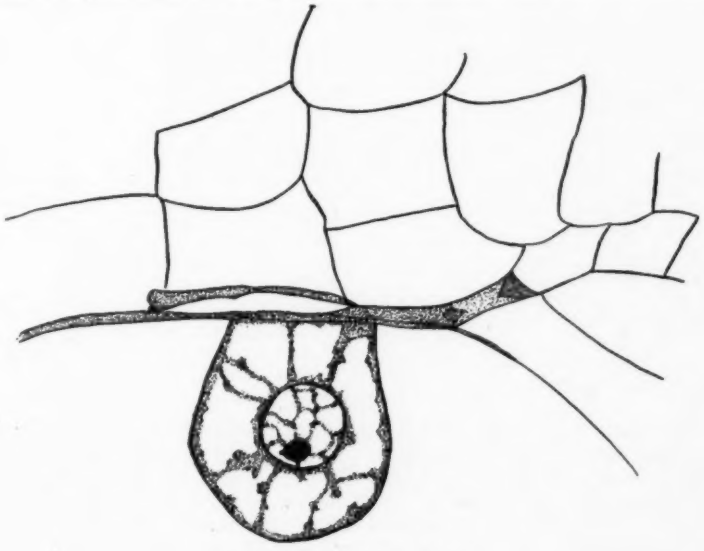

FIG. 3.—A 1 cell embryo. × 1095

VEGETATIVE STRUCTURES

The growth of the vegetative structures is gradual and continuous for about four months after pollination. The hull, shell, packing tissue, and middle septum of the nut in the early stages are similar to these respective regions, as previously described for the flower by the writers.

Hull

The hull is the modified calyx which is shed at maturity of the nut. The vascular system, sutures, color, and shape of the hull are similar from the time of pollination until the nut reaches full size in early September. An increased irregularity in the size and shape of the cells, increased evidence of tannin formation, an an increase in size and thickness of the entire region occurs during development (fig. 4*h*).

All varieties studied are divided into two natural groups on the basis of the prominence of the sutures. The thickness of the hull of varieties in group 1 is approximately the same at the sutures as midway between the sutures. The thickness of the hull of varieties in group 2 is 36.8 per cent greater at the sutures than midway between them (fig. 5). The various varieties are grouped as shown in Table 2.

Table 2.—*Thickness of hulls at sutures and between sutures, of varieties of pecans from group 1 and group 2*

Variety	Thickness of hull in millimeters—		Variety	Thickness of hull in millimeters—	
	At sutures	Between sutures		At sutures	Between sutures
Group 1			*Group 2*—Continued		
Mobile	4.34	4.34	Jerome	5.03	4.76
Nelson	4.34	4.34	Mantura	4.34	3.17
San Saba	2.12	1.90	Moneymaker	5.40	3.49
Teche	4.12	4.12	Moore	5.40	3.49
Average	3.73	3.68	Pabst	6.66	4.34
Group 2			Randal	5.08	3.17
			Russell	4.12	3.49
			Rome	6.35	4.12
Alley	4.12	2.85	Schley	5.03	4.12
Appomattox	4.76	3.81	Stuart	6.35	4.76
Atlanta	5.71	3.49	Success	3.81	2.54
Beverage	4.12	3.17	Unknown	5.08	3.49
Bradley	5.40	4.12	Van Deman	4.76	3.81
Centennial	6.35	4.76			
Frotscher	5.71	4.76	Average	5.17	3.78

Shell

The shell is the ovarian wall, located directly beneath the hull; it becomes very hard just prior to maturity and is exposed after the shedding of the hull (fig. 4, *s*). The shell is incompletely divided by the sutures into two equal segments. At a depth of about four layers of cells beneath the surface a vascular bundle extends from the base to the apex of the shell along each suture (fig. 4, *su'*). The vascular bundles which separate the hull from the shell become separated from both regions at maturity.

The color, shape, vascular system, and cell structure of the shell are similar from the time of pollination until the beginning of shell hardening, about four and one-half months later. During the period of growth the entire region increases in size and thickness, but no further growth occurs after shell hardening is well begun. The hardening begins at the apex and progresses toward the base, the regions immediately surrounding the sutures hardening in advance.

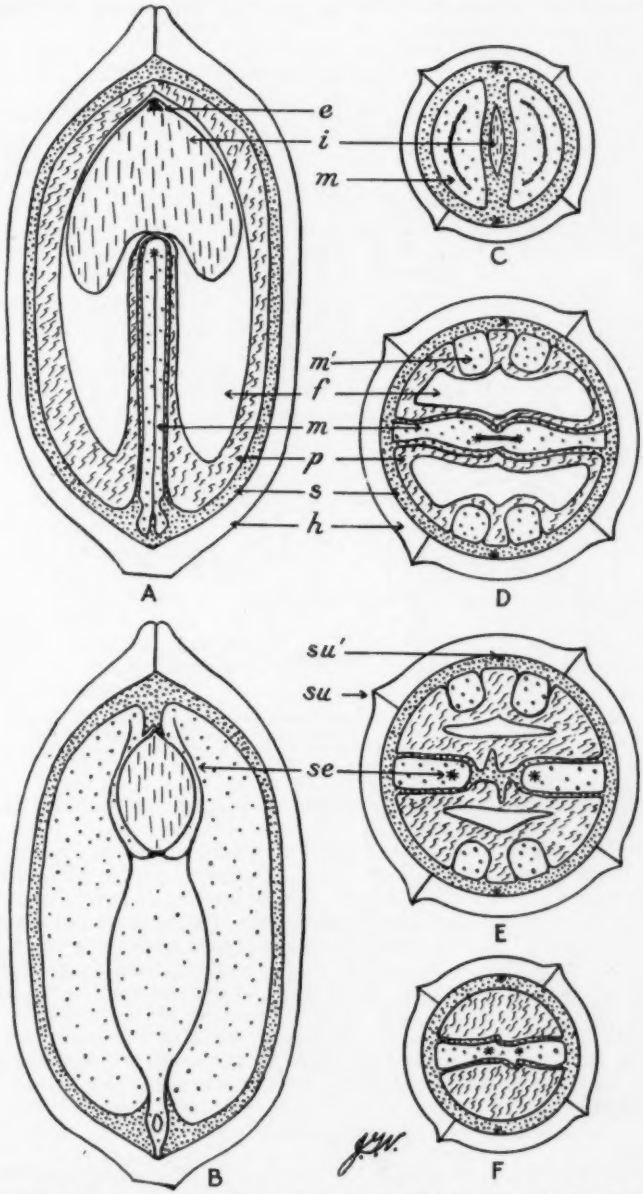

FIG. 4.—Location of the regions of the pecan nut: A, longitudinal section at right angles to the plane of the middle septum; B, longitudinal section in the plane of the middle septum; C, cross section just below the apex of the integument; D, cross section just below the opening in the middle septum; E, cross section of portion about three-fourths of the distance down from the apex; F, cross section through the lower region of the cavity of the shell; e, embryo; i, integument; m, middle septum; m', extension of the middle septum along the inner wall of the shell; f, fissure; *p*, packing tissue; *s*, shell; *h*, hull; *su*, suture of the hull; *su'*, suture of the shell; *se*, fibrovascular bundles of the middle septum.

Approximately three weeks are required for the process to progress from the apex to the base.

Middle Septum

The middle septum, with the exception of an ovoid opening near the apex, divides the ovarian cavity into two parts. At maturity it forms an indelible partition between the two halves (cotyledons) of the kernel.

The septum is composed of two types of tissue, one being partially inclosed by the other. The inner is composed of large cells, and is soft and spongy during the growing period, but crumbly, reddish-brown in color, and bitter in taste in the mature nut (fig. 4, *m*). It extends irregularly from the base to the apex of the septum, enlarging at the apex to from the cap-like structure and extending baseward along the inner walls of the shell opposite to the attachment of the septum (fig. 4, *m'*). The second type of tissue of which the septum is composed is similar in cell structure to that of the shell. Beginning at the base, it extends to the apex of the

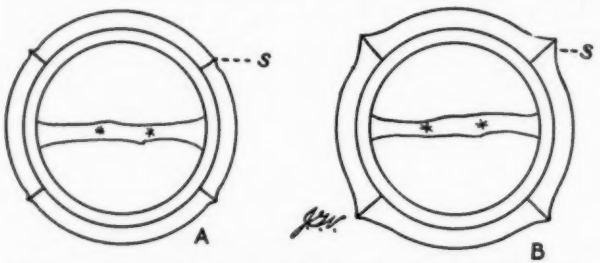

Fig. 5.—A, cross section of pecan hull from group 1, which, as is characteristic of the group, is approximately the same thickness at all points; B, cross section of a hull from group 2, which, as is characteristic of this group, is thicker at the sutures than midway between the sutures

embryo and forms a thin protective covering for the more delicate inner tissue of the septum.

The vascular bundles of the middle septum are embedded in the inner tissue (fig. 4, *se*). They enter through the base of the shell at two points. After entering the nut they curve outward from one another to come slightly inward again near the base of the opening in the septum. At this point a branch from each converges and enters the seed coat at the base of the opening, forming a complex system of vascular bundles. The point of entrance of the bundles of the septum into the seed coat is marked at maturity by an elliptical, slightly raised scar. The main bundles curve around the opening and continue toward the apex of the shell, terminating just before they reach the upper limits of the tissue in which they are embedded.

Packing Tissue

The remaining space within the young nut is filled with a white, dry, soft, spongy tissue that is present at the time of pollination but does not become distinct until several weeks later. It fills all space within the shell that is not occupied by the middle septum and has

the outline of the kernel of a completely filled nut (fig. 4, *p*). The crevice or fissure, mentioned by the writers in the description of the flower, becomes wider and deeper as the nut grows, so that a cavity or pocket is formed in the packing tissue on either side of the septum to accommodate the expanding seed coat (fig. 4, *f*). As the seed coat enlarges and fills the shell, the packing tissue is pressed closely against the walls of the shell and middle septum and at maturity forms a layer of dry, brittle material.

The packing tissue does not have a vascular system and is in a state of constant change, due to the growth and filling of the seed coat by the embryo. The cells bordering on the fissure are smaller than those composing the main body of the tissue.

If the embryo, and consequently the seed coat, does not completely fill the cavities of the shell, the packing tissue will not be pressed firmly against the walls; in which case a part of the tissue will cling to the seed coat at maturity as dry, brown, spongy fragments. Such nuts are popularly known as "chaffy" nuts.

REPRODUCTIVE STRUCTURES

Integument or Seed Coat

The ovule is about two-thirds inclosed by the integument at pollination time and is completely inclosed 10 to 15 days later. Growth and expansion is slow during the following two and one-half months. About July 15 the rate of growth of the integument, or capsule, increases. It expands into the fissures of the packing tissue (fig. 4, *i*), forces the latter against the surrounding walls, and reaches full size a month later. On becoming fully grown the integument becomes the seed coat, incloses the growing embryo, and soon assumes a definite and permanent shape (fig. 6, *a* and *b*).

The irregularities in the outline of the seed coat at maturity are due to the four projections from the caplike structure along the walls of the shell (described under middle septum) and to the unevenness in the surfaces of the middle septum. The seed coat of well-filled varieties, such as the Schley and Curtis, have a smoother surface at maturity than varieties not so well filled, such as the Frotscher and Teche.

An elliptical scar corresponding to that found at the base of the opening of the middle septum is present on the seed coat at the point of its attachment to the middle septum. Radiating from this scar are numerous vascular bundles which branch profusely and extend throughout the seed coat. The color of these bundles both before and after maturity is similar to that of the seed coat.

Embryo Development or "Filling"

The nucellus, with the small embryo sac, fills the integument during the early stages of development. Active cell division has been observed during the first few weeks after pollination, but how long it continues was not determined. However, the nucellus is later absorbed as food material for the developing embryo. The embryo sac reaches a rather large size before the embryo begins growth.

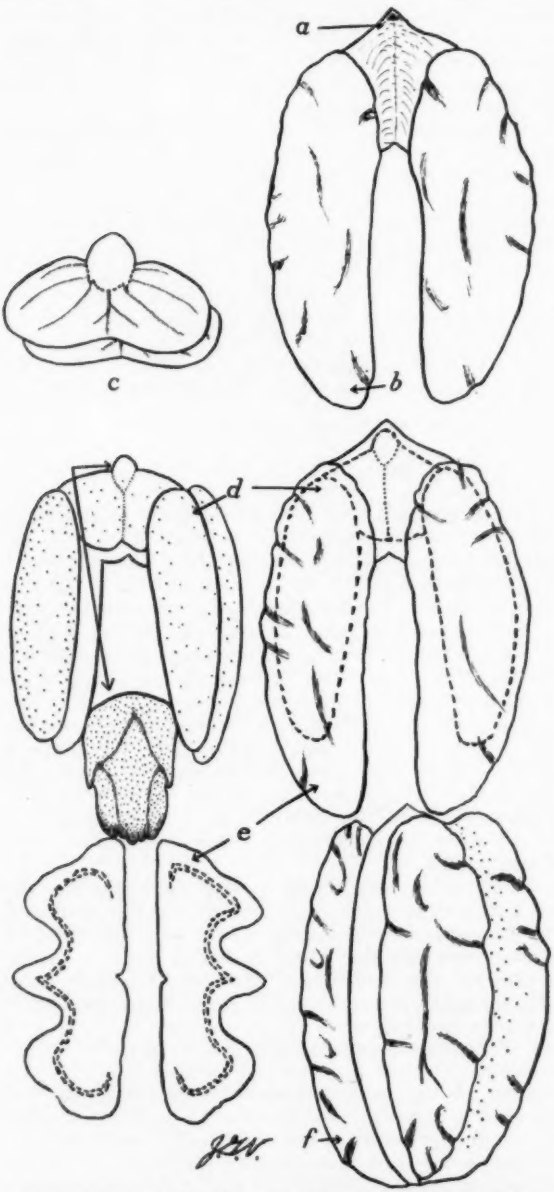

FIG. 6.—Manner of filling of the pecan nut: *a*, a young embryo about the second week in August; *b*, the seed coat, which was fully developed when filling began; *c*, the embryo about the last week in August; *d* and *e*, the rapidly developing cotyledons within the seed coat; *f*, the fully developed embryo about October 10

The seed coat enlarges much more rapidly than the embryo, the additional space being filled by a semiclear fluid.

During the earlier part of the development period considerable pressure is exerted by this fluid, thus forcing the seed coat outward and downward into all available space within the shell. (Fig. 4, *i*.) The pressure becomes less after the embryo fills about half of the interior of the seed coat.

Beginning with the 64 cell embryo (fig. 6, *a*, *b*, *c*, *d*, *e*, and *f*), previously mentioned as being present 12 weeks after pollination rapid growth follows for about five weeks. Two double winglike appendages (the cotyledons) develop on each side of the young embryo (fig. 6, *c*), growing sideways until the edges reach the walls of the seed coat, which causes them to turn toward the base. Expansion is first in length and width, then in thickness (fig. 6). Included within the folds of each cotyledon is a thin white membrane containing numerous vascular bundles. Previous to the thickening of the cotyledons this membrane is a thick succulent pad of material. The origin and purpose of this membrane has not been determined (fig. 7, *b*). Table 3 shows the varieties of pecans used in this work, as well as the relative stages of development of the seed coat, shell hardness, embryo development, and maturity, with the dates on which these occur.

TABLE 3.—*Seed-coat development, shell hardness, and embryo development at different periods, and dates of maturity, for different varieties of pecans*

Variety	Condition on Aug. 13 of—			Condition on Sept. 4 of—						Date of maturity in—	
	Seed coat development, 1926	Shell hardness, 1926	Embryo development, 1926	Seed coat development			Shell hardness		Embryo development, 1926	1924	1926
				1924	1925	1926	1925	1926			
	Per cent			*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*		
Alley	20	Not hard	Not visible	15	100	100	5	50	5	Nov. 11	
Appomattox	15	do	do	15	50	100	30	60	20	Nov. 15	Nov. 6
Atlanta	15	do	do	60	15	100	5	30	0	Nov. 10	
Beverage	50	do	do	100	50	100	40	100	20	Oct. 15	Nov. 10
Bradley	10	do	do	10	30	90	5	15	0	Nov. 15	
Centennial	90	10 per cent	do	100	70	100	90	100	100	Oct. 10	Oct. 17
Curtis	30	Not hard	do	10	50	100	5	30	15	Nov. 10	
Delmas							5	10			
Frotscher	25	Not hard	Not visible	10		100		10	0	Oct. 25	Oct. 30
Indiana	90	50 per cent	do		50	100	90	100	100	Oct. 5	
Jerome	90	40 per cent	do	100	90	100	70	100	90	Oct. 10	Oct. 4
Mantura	65	Not hard	do	100	70	100	30	100	30	Oct. 15	Oct. 17
Mobile	30	do	do	60	50	80	60	10	0	Nov. 15	Nov. 6
Moneymaker	90	10 per cent	do	100	70	100	90	100	50	Oct. 15	Oct. 4
Moore	80	5 per cent	do	60	70	100	5	100	50	do	Oct. 17
Nelson	30	Not hard	do	15		100		100	15	Nov. 11	Oct. 30
Pabst	60	do	do	20	90	100	5	90	20	Oct. 28	Nov. 6
Randal	30	do	do	15	15	100	5	10	0	Nov. 10	Nov. 12
Rome	90	50 per cent	do	100	90	100	90	100	90	Oct. 10	Oct. 4
Russell No. 3	90	5 per cent	do	65	15	100	5	90	15		Nov. 10
Russell				15	15	100	5				
San Saba	60	5 per cent	Not visible	65	50	100	10	90	50	Oct. 20	Do.
Schley	60	do		15	70	100	5			Oct. 15	Oct. 17
Stuart	60	do	Not visible	100	35	100	5	100	90	Oct. 18	Oct. 29
Success	60	do	do	70	70	100	75	100	5	Oct. 15	Oct. 4
Teche	25	Not hard	do	15	70	90	50	10	0	Nov. 11	Oct. 30
Unknown	60	5 per cent	do			100		80	20	Oct. 20	
Van Deman	60	do	do	15		100		30	0	Oct. 15	
Waukenah							5	10			
Average	54.4	7.7	0	50	52.2	98.5	31.6	63.9	31	Oct. 25	Oct. 25

The Process of Ripening

The process of ripening includes those chemical and physical changes which take place within the nut from the time the embryo reaches full size until it is palatable.

Chemical Changes

Nine samples were taken for chemical analyses. The first seven were taken on successive weeks, beginning just before the seed coat was fully filled; two weeks elapsed between the time of taking the seventh and eighth sample, and five weeks between the time of taking the eighth and ninth. The nuts were ready for harvest at the time of the taking of the eighth sample. The Bradley and Mobile varieties, both of which mature very late in the season, were used. Fifty nuts constituted a sample. The nuts were hulled, shelled, dried at 70° C., and analyzed according to the methods used by the American Association of Official Agricultural Chemists (*1*). The results of the analyses are shown in Table 4.

There was a progressive change in the composition of the embryo until the time of taking the fourth sample, or four weeks before the time of harvest. However, after the fourth sample was taken the composition was fairly constant. At this time the color markings were faintly visible at the tip of the shell. Two weeks later the abscission of the shell and hull began and continued for two weeks, accompanied by the drying of the tissues of all the vegetative regions.

Table 4.—*Chemical changes which take place within the pecan nut during the growing period, as indicated by analyses*

BRADLEY VARIETY

Date of record	Weight of fresh sample in grams	Percentage of dry matter	Total weight of dry matter in grams	Ash		Protein (N×6.25)		Fiber		Nitrogen-free extract		Fat	
				Per cent	Grams	Per cent	Grams	Per cent	Grams	Per cent	Grams	Per cent	Grams
Sept. 25	112.7	24.99	28.16	4.66	1.31	13.68	3.85	5.40	1.52	28.02	7.89	48.23	13.58
Oct. 4	143.5	29.01	41.63	2.82	1.17	12.03	5.01	5.72	2.38	19.03	7.92	60.40	25.14
Oct. 11[1]	166.0	38.01	63.10	2.95	1.86	15.66	9.88	5.12	3.23	16.38	10.34	59.89	37.79
Oct. 18	146.0	62.46	91.20	2.03	1.85	11.20	10.21	3.17	2.89	16.17	14.75	67.43	61.50
Oct. 25	149.0	65.53	97.65	1.80	1.76	11.49	11.22	3.55	3.47	16.18	15.80	66.98	65.41
Nov. 1	162.0	69.43	112.48	1.74	1.96	11.23	12.63	3.86	4.34	15.95	17.94	67.22	75.61
Nov. 8	172.0	70.78	121.75	1.65	2.01	11.75	14.31	3.41	4.15	15.59	18.98	67.61	82.32
Nov. 22	-------	-------	-------	1.66	-------	11.49	-------	2.82	-------	16.28	-------	67.76	-------

MOBILE VARIETY

Sept. 25	101.4	20.29	20.58	4.49	0.92	17.15	3.53	4.55	0.94	23.48	4.83	50.33	10.36
Oct. 4	155.0	42.77	66.30	2.55	1.69	13.82	9.16	3.60	2.39	20.73	13.74	59.30	39.32
Oct. 11	90.5	55.42	55.14	2.07	1.14	12.62	6.96	3.60	1.99	15.07	8.31	66.64	36.75
Oct. 18	144.0	60.18	86.67	1.83	1.59	13.26	11.49	2.37	2.05	16.65	14.43	65.89	57.11
Oct. 25	170.0	61.47	104.50	1.81	1.89	13.09	13.68	2.95	3.08	15.57	16.27	66.58	69.58
Nov. 1	171.0	65.46	111.95	1.72	1.93	13.34	14.93	2.49	2.79	14.23	15.93	68.22	76.37
Nov. 8	180.0	64.91	116.85	1.79	2.09	13.51	15.79	2.84	3.32	13.85	16.18	68.01	79.47
Nov. 22	-------	-------	-------	1.96	-------	13.94	-------	2.89	-------	13.38	-------	67.82	-------

[1] Teche variety.

From the table it will be noted that the dry matter of the embryo increased from 28.16 grams to 121.75 grams, and from 20.58 grams to 116.85 grams, respectively, for the two varieties. The first four

weeks were marked by an increase in weight of dry matter and percentage of oil and a decrease in percentage of ash, fiber, nitrogen-free extract, and protein. During the period covered by the last five analyses the weight of dry matter and the percentage of oil increased

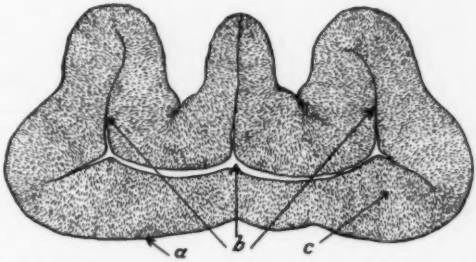

FIG. 7.—Cross section of pecan cotyledon: *a*, semipermeable membrane inclosing the entire kernel; *b*, transparent membrane in the center of the cotyledon; *c*, intermembrane tissue, practically uniform in structure

slightly, while the ash, fiber, protein, and nitrogen-free extract remained almost constant. In total weight there was an increase in ash, protein, fiber, nitrogen-free extract, and fat.

The decrease in the percentage of nitrogen-free extract is due to a decrease in soluble carbohydrates. The decrease in percentage of fiber was probably due to a decrease in the ratio of the seed coat to embryo as the seed coat was filling. The increase in percentage of oil is due to the transformation of carbohydrates into oil.

FIG. 8.—A, full-grown nut, Pabst variety, showing the sutures of the hull; B, hull opened along sutures, and nut ready for harvest; C, nut showing color "markings"

MATURITY

Maturity may be said to begin as soon as all portions of the nut reach full size, from five to six weeks before harvest. All of the regions begin to lose moisture and to change in color, beginning at the apical end and progressing baseward. Simultaneously with the ripening of

both hull and shell, as evidenced by the change in color, the hull divides into four nearly equal segments and separates from the shell, after which the nuts are ready for harvest (fig. 8.) The changes in color of the various regions of the nut during the process of ripening (*4*) are shown in Table 5:

TABLE 5.—*Color of the various regions of the pecan nut before maturity and when fully matured*

Region	Color before maturity	Color when fully mature
Hull	Pale green oxide of chromium	Greenish-black or cedar green.[1]
Shell	Pale gray green	Putty color.
Shell lining	Snow white	Dark fawn.
Packing tissue	do	Blood-red brown.
Seed coat	Amber white	Honey yellow.
Embryo	Snow white	Creamy white.

[1] If for some reason the hull and shell do not separate normally, the hull will be lighter in color at maturity.

Due to many causes, nuts do not mature normally. The presence of the shuck worm in the hull region, premature frosts, the mechanical severing of the nut from the tree, or other conditions which interfere with the function of any or all regions, will prevent the hull from dividing into segments and separating from the shell. The failure of the hull and shell to separate is an indication that the nut is partially or totally unfilled.

COLOR MARKINGS

The formation of the typical color markings on the shell of the pecan is a result of the process of separation of the hull from the shell. The vascular system between the hull region and the shell region becomes separated and is set almost free from both shell and hull. Normally, when the nut is removed this vascular material remains in the hull, but with care it may be removed with the shell. From two to five weeks before the nut is mature the middle lamellae of the cells of the hull surrounding the vascular bundles undergo changes which allow them to separate. The exact time or period when these cells are set aside as an abscission layer has not been determined, but examination of scrapings from the tissue about the bundles and from the color markings on the shell indicate that the color markings are the result of a deposit of the separated cells of the abscission layer. They appear as a single layer of large thin-walled cells.

The markings on the mature nut appear as dots or streaks. The streaks more commonly appear toward the apical end of the nuts, while the basal end has dots only. The relative number of dots and streaks on an individual nut is a varietal character. The Russell, Bradley, Curtis, Atlanta, and Centennial varieties have a large number of dots, while the Rome, Frotscher, and Alley varieties have numerous streaks and few dots. The markings may be removed from the shell by rubbing or brushing. The Frotscher, Van Deman, Mobile, and other varieties frequently lose a large part of the markings during the harvesting process.

Nuts from which the hulls were removed before ripening began failed to develop markings (fig. 9, B); nuts from which the hull was removed after the ripening process started but before it was com-

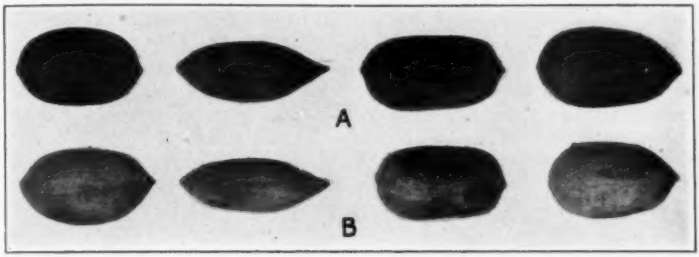

FIG. 9.—A, nuts with typical color markings; B, nuts without color markings as the result of girdling the shell a few weeks before maturity. Varieties, reading from left to right, Delmas, Atlanta, Pabst, and Pabst

pleted developed faint markings; and nuts from which one-half of the hull was removed before ripening, leaving the remainder intact, failed to develop markings where the shell was prematurely exposed.

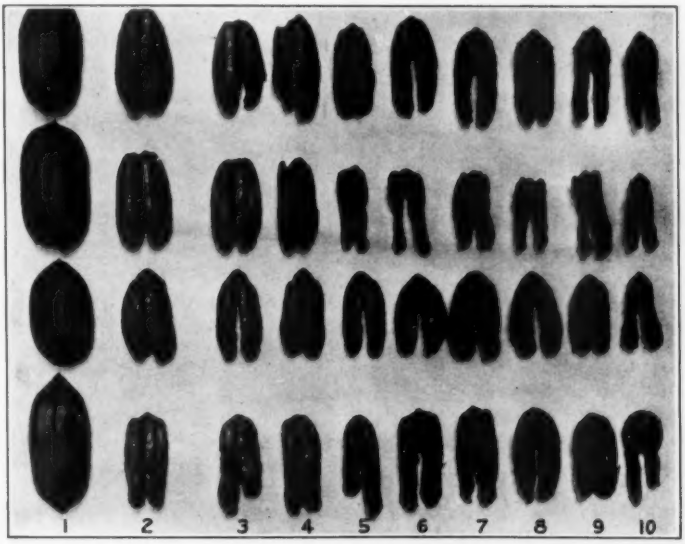

FIG. 10.—Comparison of good and faulty nuts: Column 1, left to right, mature kernels in the shell; column 2, fully developed kernels; columns 3 to 10, inclusive, kernels showing various degrees of faultiness Varieties by rows, reading from top to bottom, Schley, Pabst, Teche, and Mobile. The Mobile is the only variety that does not have fully developed seed coat in every case

FAULTY NUTS

Faulty nuts (fig. 10) are found in all varieties. The term is applied to nuts with seed coats partially or wholly unfilled at maturity. The term may also be applied to nuts that were normal at maturity but were later attacked by insects or fungi. It is with those nuts

whose seed coats are partially or wholly unfilled at maturity that this study is most concerned because this condition is due to the activity within the nut itself, as contrasted with the condition of nuts attacked by insects or fungi, which are outside agencies.

Faultiness is correlated with other activities, either of the entire tree or some part of the tree, and may be traced to direct causes. In some cases it is traced to extremely dry weather which so interferes with the growth of the tree that it can not support nut development at the time the embryo is making rapid growth. Among prolific varieties, as the Mobile and Teche, a heavy crop may so tax the resources of the tree that filling of the nuts can not take place properly. Wide areas along the Gulf Coast produced faulty nuts in 1926 because of a storm which occurred September 20 defoliated the trees, and thus prevented photosynthetic activities. A very late variety, such as the Bradley or Randal, often produces a high percentage of faulty nuts as the result of early frost, which checks growth before filling is completed. Nuts were rendered faulty mechanically by girdling the hull region of varieties in various stages of embryo development. This has a direct result on the condition of the nuts at maturity. The effects of girdling on nuts are shown in Table 6.

TABLE 6.—*Effect of girdling on nuts, at various stages of their development*

Variety	Number of nuts treated	Condition of nuts when girdled	Condition of nuts at maturity
Rome	10	Shell hard; seed coat filled	All normally filled.
Mobile	20	Shell hard; seed coat 30 per cent filled	Poorly filled.
Atlanta	20	do	Do.
Teche	20	Shell hard; seed coat 20 per cent filled	Poorly filled; poor flavor.
Mobile	10	Shell partly hard; seed coat unfilled	Four partly filled; six unfilled.
Beverage	20	Shell partly hard; seed coat 5 per cent filled	Practically no filling.
Bradley	10	Shell partly hard; seed coat undeveloped	100 per cent drop.

Girdling nuts of the Rome variety after the seed coat was filled did not produce faulty nuts; girdling nuts of the Mobile, Atlanta, and Teche varieties which were partly filled produced faulty nuts; girdling nuts of the Bradley and Beverage varieties that were unfilled produced all faulty nuts. Faultiness has not been found to be related to the size or shape of the nut.

From a lot of nuts of the Pabst variety, those that had developed faint color markings were selected. The cracking strength of the shell and the filling was noted as is shown in Table 7.

TABLE 7.—*Relation of color markings and cracking strength of pecan nuts to faultiness, Pabst variety*

Condition of color markings	Number of faulty nuts	Number of good nuts	Pressure required to crack nuts (in pounds)
Very faint	109	4	61.0
Normal	6	110	68.2

Assuming these figures as typical of a large number of varieties, it may be stated that faintness of color markings is directly related to faultiness, and that low cracking strength is somewhat related to faultiness.

SUMMARY

Fertilization in *Hicoria pecan* Brit. occurs from 5 to 7 weeks after pollination, but the embryo does not become plainly visible until 10 weeks after pollination.

The pecan nut is made up of six distinct regions, viz, the hull, the shell, the middle septum, the packing tissue, the seed coat, and the embryo, the first four of which are purely vegetative.

The factors which influence the size of the nut operate during the early and middle part of the growing season, and those which influence "filling" operate during the latter part of the growing season.

Faulty nuts and undeveloped color markings are correlated, both conditions having been produced artificially.

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SULPHURIC ACID AS A WEED SPRAY[1]

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HISTORICAL REVIEW

EARLY WORK

One of the most striking methods of weed control is the use of selective sprays which will kill the weeds without injury, or with but slight injury, to the growing crop. These sprays are used for killing annual weeds in grain crops. When this method was first accidentally discovered, in 1896, by the French grape grower, L. Bonnet, it aroused great interest throughout the agricultural world, with the result that in the following years more or less extensive experiments were carried out.

In the United States Bolley (*7*),[3] who was the earliest worker to try out weed sprays, and he obtained excellent results. He states: "Farmers have been backward in applying what the writer believes to be one of the most effective methods for aiding the cereal producer in eradicating weeds." So successful were Bolley's experiments that he wrote: "The gain to the country at large * * * will be much greater in monetary consideration than that which has been afforded by any other single piece of investigation applied to field work in agriculture." However, after his experience in 1900 Bolley (*6*) changed his opinion to some extent. That year was dry, and it was shown that "these weeds [mustards, etc.] do not always die down by the treatment so easily, indeed that in dry slow-growing periods spraying should not be attempted." Bolley used several sprays, including soldium chloride, iron sulphate, copper sulphate, corrosive sublimate, and sodium arsenite.

Several other workers, Pammel and King (*28*), Moore and Stone, (*23*), Stone, (*36*), and others obtained similar results. The success of the spraying was connected rather closely with prevailing conditions for growth and weather during and after the spray had been applied. Schultz (*34*), Bornemann (*8*), Wehsarg (*39*), and other German authors described extensive experiments with various chemical sprays in Germany, where the method was utilized more than in any other part of the world. Extensive experiments are also reported from England and Scotland, since workers were very eager to try the new method of weed eradication. Brenchley (*9*) summarizes several of these experiments.

Many countries now possess a rather voluminous literature dealing with weed sprays. Sulphate of iron was the chemical used chiefly

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[2] The writer is deeply indebted to Prof. W. C. Muenscher, who has given many helpful suggestions during the planning and progress of these experiments. An award of a fellowship from the International Education Board made it possible for the writer to undertake this investigation.

[3] Reference is made by number (italic) to "Literature cited," p. 1089.

or exclusively. The results obtained are various and often contradictory. Under favorable conditions the results were excellent, but failures caused by rains or other complications are often reported.

A NEW PERIOD

In the earlier experiments various chemicals, usually solutions of salts, were used as sprays. Acids do not seem to have been tried to any great extent, and when used the results seem to have been no more satisfactory than with other sprays. For instance, Woods (*41, p. 100–101*) in 1904, reports that a 20 per cent solution of iron sulphate reinforced with 5 per cent sulphuric acid failed to destroy *Raphanus raphanistrum*. Experience indicates that *R. raphanistrum* is very easily killed by either sulphuric acid or iron sulphate. It is possible that Woods was working with another similar plant, possibly *Brassica campestris*.

However, in 1911 Rabaté (*29*) reports some results from experiments on weed eradication in winter wheat, which mark the beginning of a new period. He used solutions of copper sulphate, iron sulphate, and sulphuric acid. He concludes that sulphuric acid in 6, 8, or 10 per cent solutions, the degree of strength depending upon local conditions, is the most satisfactory spray to use. The solution was applied at a rate of 1,000 liters per hectare (107 gallons per acre). It killed most annual weeds but did not retard the growth of the wheat, although the lower leaves were killed. The sulphuric acid solution also had a fertilizing effect on the soil. Later on Rabaté (*30, 31*), as well as other workers in France, reported similar results. Jaguenaud (*18*) found that sulphuric acid killed wild radish, wild mustard, crowfoot, vetches, and vetchling without injury to wheat. He used 7 liters of sulphuric acid (66° Baumé) to every hectoliter of water, which gave approximately a 10 per cent solution. Several papers deal with the eradication of weeds in flax (*17, 19, 32*). Morettini (*24*) in 1914–15 reports similar results in Italy. In sprayed fields he obtained an increase of 6 bushels of grain per acre.

In the Scandinavian countries, especially in Norway, the new method of weed eradication is now extensively used. Korsmo (*20, 21, 22*) started very comprehensive experiments on weed eradication in 1914. The chief spray he used was a diluted sulphuric acid. In spring-sown grain he found a strength of 3.5 to 4 per cent solution applied at a rate of 1,000 liters per hectare (107 gallons per acre), sufficient to kill all annual weeds to which the spray could adhere. As a result he obtained a very marked increase in yield. For instance, for the average of 211 experiments carried out from 1914 to 1922 in spring-sown grain crops, he obtained an increase of 490 kgm. of grain (25.3 per cent above unsprayed plots) per hectare. Calculated per acre the increase in yield of grain would be about 430 pounds.

Unfortunately, the results are published only in Norwegian, so that they are almost unknown outside of Scandinavia. In 1921 the writer (*1, 2, 4*) began experiments on weed eradication in Sweden under conditions similar to those of Korsmo, and has obtained very satisfactory results.

WEEDS REPORTED KILLED BY SULPHURIC ACID

Table 1, listing weeds reported killed by sulphuric acid, is compield from the reports of several workers.

TABLE 1.—*Weeds reported killed by sulphuric acid solution of different concentrations*

Name of weed	Investigator reporting	Percentage concentration of sulphuric acid solution used
Adonis aestivalis L. (summer adonis, or pheasant's-eye)	Morettini	10.0
Anthemis arvensis L. (corn camomile)	Korsmo	3.5 to 4.0
Anthemis cotula L. (May weed or dog fennel)	do	3.5 to 4.0
	Rabaté	10.0
Brassica alba (L.) Boiss. (white mustard)	Korsmo	3.5 to 4.0
Brassica arvensis (L.) Ktze. (wild mustard or charlock)	Korsmo and others	3.5 to 10.0
Brassica campestris L. (rutabaga)	Korsmo	3.5 to 4.0
Capsella bursa-pastoris Med. (shepherd's purse)	do	3.5 to 4.0
	Rabaté	10.0
Centaurea cyanus L. (bachelor's button)	Korsmo	3.5 to 4.0
Chenopodium album L. (pigweed, lamb's quarters)	Aslander	3.5 to 4.0
Chenopodium rubrum L. (red goosefoot)	Korsmo	3.5 to 4.0
Chenopodium polyspermum L. (pigweed or lamb's quarters)	do	3.5 to 4.0
Chrysanthemum segetum L. (yellow oxeye)	do	3.5 to 4.0
Cuscuta sp. (dodder)	Rosa (33)	4.0 to 5.0
Daucus carota L. (wild carrot)	Morettini	10.0
Delphinium consolida L. (larkspur)	Korsmo	3.5 to 4.0
Erophila verna E. Mey	Rabaté	10.0
Erysimum cheiranthoides L. (wormseed mustard)	Korsmo	3.5 to 4.0
Euphorbia peplus L. (petty spurge)	do	4.0
Fagopyrum tartaricus Gaertn. (Tartarian buckwheat)	do	3.5 to 4.0
Galeopsis tetrahit L. (hemp nettle)	do	3.5 to 4.0
Galeopsis speciosa Mill	do	3.5 to 4.0
Galium aparine L. (cleavers)	do	3.5 to 4.0
Lamium amplexicaule L. (henbit)	do	3.5 to 4.0
Lamium purpureum L. (dead nettle)	do	3.5 to 4.0
Lapsana communis L. (nipplewort)	do	3.5 to 4.0
Lathyrus spp. (wild peas)	Jaguenaud	10.0
Lepidium campestre (L.) R. Br. (field peppergrass)	Korsmo	3.5 to 4.0
Lepidium ruderale L. (peppergrass)	do	3.5 to 4.0
Matricaria discoidea DC. (pineappleweed)	do	3.5 to 4.0
Matricaria chamomilla L. (wild chamomile)	Jaguenaud	10.0
Papaver argemone L.	Korsmo	3.5 to 4.0
Papaver dubium L. (poppy)	do	3.5 to 4.0
Papaver rhoeas L. (field poppy)	do	3.5 to 4.0
	Rabaté	10.0
Polygonum aviculare L. (knotweed, or knotgrass)	Korsmo	3.5 to 4.0
Polygonum convolvulus L. (black bindweed, or wild buckwheat)	do	3.5 to 4.0
Polygonum lapathifolium Ait. (smartweed)	do	3.5 to 4.0
Pteris aquilina L. (bracken, or brake fern)	Gordon (14)	5.0
Ranunculus arvensis L. (buttercup)	Rabaté	10.0
	Morettini (25)	10.0
Ranunculus ficaria L. (lesser celandine)	Rabaté	10.0
Ranunculus spp. (buttercups)	Jaguenaud	10.0
Raphanus raphanistrum L. (wild radish)	Korsmo	3.5 to 4.0
Senecio vulgaris L. (groundsel)	do	3.5 to 4.0
Sisymbrium officinale (L.) Scop. (hedge mustard)	do	3.5 to 4.0
Sisymbrium sophia L. (flixweed)	do	3.5 to 4.0
Solanum nigrum L. (black nightshade)	do	3.5 to 4.0
Sonchus oleraceus L. (sow thistle)	do	3.5 to 4.0
Specularia speculum Alph. DC. (Venus's-looking-glass)	Morettini	10.0
Spergula arvensis L. (corn spurry)	Korsmo	3.5 to 4.0
Stellaria media (L.) Cyrill. (chickweed)	do	3.5 to 4.0
Thlaspi arvense L. (penny cress, French weed)	do	3.5 to 4.0
Urtica urens L. (nettle)	do	3.5 to 4.0
Vicia spp. (vetches)	Jaguenaud	10.0
Viola tricolor (hearts ease)	Korsmo	3.5 to 4.0

Table 1 shows a wide range of concentration of sulphuric acid used. Korsmo never used more than a 4 per cent solution and obtained excellent results. In France and Italy a 10 per cent solution is commonly claimed to be necessary for the complete destruction of the weeds. This difference probably is due chiefly to the fact that Korsmo has been working in spring-sown crops, while the later data are obtained in fields of winter wheat sprayed in February or March. Rapidly growing plants in spring-sown crops are much more sus-

ceptible than winter annuals in wheat. This point will be fully discussed later. This apparent difference in the susceptibility of plants may also be due in part to the different types of spraying machines employed. Korsmo had a machine constructed especially for spraying sulphuric acid, which worked very satisfactorily. The possibility of killing a certain weed by a dilute sulphuric-acid solution depends on the amount of spray actually adhering to the plant, which in turn depends to a great extent upon the type of spraying machine used. The writer's experiments on weed eradication have confirmed the importance of a good spraying machine in obtaining good results. That the importance of the proper spraying machine has sometimes been overlooked is clear when a writer recommending a 10 per cent solution declares that the spray can be distributed by a watering can.

WEEDS REPORTED BADLY INJURED BY SPRAY OF SULPHURIC ACID

Certain weeds are not killed by sulphuric-acid spray. Among these are many perennial weeds the leaves of which may be destroyed while the roots are unharmed, thus allowing new shoots to appear soon. Spraying of growing crops is not directed against perennial weeds but against annual, winter annual, and possible biennial weeds. Table 2, which lists some of the weeds reported badly injured by sulphuric-acid spray does not therefore, embrace all weeds known to be harmed. This does not mean that sprays, especially sulphuric-acid sprays, may not be of some importance in combating some perennial weeds. For instance, it is the writer's experience that spraying an oat field at the proper time will prevent shoots of *Cirsium arcense* (Canada thistle) from flowering. Dehn (*13*) states that the best method of eradicating weeds in lawns is to apply some drops of sulphuric acid on the crown of each plant.

TABLE 2.—*Weeds reported badly injured by spraying with sulphuric-acid solution*

Name of weed	Investigator reporting	Percentage concentration of sulphuric acid solution used
Adonis flammea Jacq	Rabaté	10
Agrostemma githago L. (corn cockle)	do	10
Centaurea cyanus L. (bachelor's button)	do	10
Lathyrus aphaca L. (wild pea)	do	10
Lathyrus hirsuta L. (wild pea)	do	10
Scandix pecten-veneris L.	do	10
Vicia angustifolia Reichard (vetch)	do	10
Vicia cracca L. (wild vetch)	do	10

WEEDS NOT INJURED BY SPRAYS OF SULPHURIC ACID

Several weeds are reported as not injured by sulphuric acid. A waxy surface and the concealing of the easily injured growing point protects the grasslike weeds as well as the grain plants. Dense hairs or glandular hairs protect some weeds against the sulphuric acid spray as well as against other sprays which act on the top of the plants. Several weeds have a smooth surface to which the spray can not adhere.

TABLE 3.—*Weeds reported not injured by spraying with sulphuric-acid solution*

Name of weed	Investigator reporting	Percentage concentration of sulphuric-acid solution used
Allium rotundum L. (wild onion)	Morettini	10.0
Allium vineale L. (wild garlic)	do	10.0
	Voelcker (37)	
Alopecurus agrestis L. (foxtail grass)	Rabaté	10.0
Anchusa officinalis L. (alkanet)	Korsmo	3.5 to 4.0
Arena fatua L. (wild oats)	Several workers	3.5 to 10.0
Arena strigosa Schreb.	Korsmo	3.5 to 4.0
Bromus mollis L.	do	3.5 to 4.0
Bromus secalinus L. (chess or cheat)	do	3.5 to 4.0
Carduus crispus L.	do	3.5 to 4.0
Cirsium lanceolatum Scop. (bull thistle)	do	3.5 to 4.0
Erodium cicutarium L'Her. (stork's-bill)	do	3.5 to 4.0
Euphorbia helioscopia L. (wartweed)	do	3.5 to 4.0
Fumaria officinalis L. (fumitory)	do	3.5 to 4.0
Lolium temulentum L. (darnel)	do	3.5 to 4.0
Matricaria inodora L.	do	3.5 to 4.0
Medicago spp.	Morettini	10.0
Muscari spp. (grape hyacinth)	do	10.0
Ornithogalum spp.	do	10.0
Senecio viscosus L.	Korsmo	3.5 to 4.0
Sonchus asper L., Hill	do	3.5 to 4.0

Tables 1 to 3, inclusive, show that most weeds, not perennial, are killed when sprayed with sulphuric acid. Those unharmed are comparatively few and generally not so troublesome. These tables are not intended, however, to give the impression that sulphuric acid is the only spray which will kill these weeds. Under favorable conditions a solution of iron sulphate or copper sulphate may produce just as good results. Reports of experiments show, nevertheless, that the latter sprays often fail. Some of the reasons for this failure will be considered later in this paper.

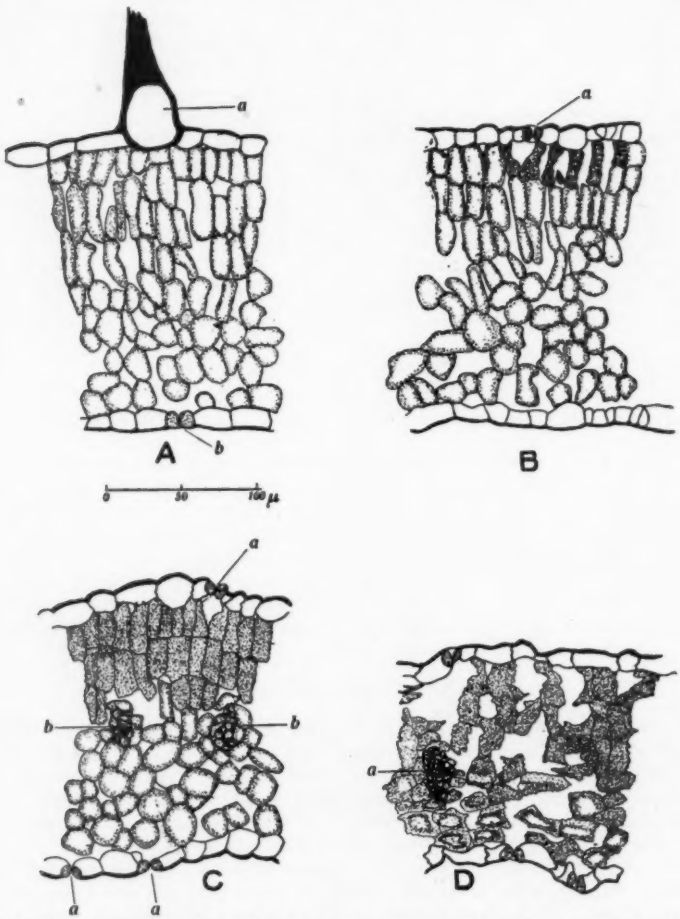
PREVIOUS EXPERIMENTAL WORK

In hundreds of field trials it has been demonstrated that dilute sulphuric acid is an excellent spray. However, the action of the acid on plant tissues seems not to have been studied to any great extent. Recently the writer (*3*) has been able to show how sulphuric acts upon plants. Since a knowledge of this action is necessary for a further discussion, a brief summary is given here.

Several weeds and crop plants, grown in crocks in the greenhouse, as well as *Brassica arvensis* (mustard) growing in the field, were sprayed with diluted sulphuric acid solutions of various strengths. Because they were so hardy that they were not harmed by weak sprays the action of the sprays on mustard plants growing in the field was very easily studied. Cross sections of the sprayed and unsprayed leaves were made by the paraffin method. These sections were then examined microscopically to determine the action of the sulphuric acid upon the tissues of the leaves. The most striking action of the sulphuric-acid spray was as follows:

(1) After adhering to the surface of the plants it penetrates very rapidly and kills the protoplasm almost instantly. Leaves of mus-

tard have stomata on both sides (fig. 1, A). It was found that the acid at first penetrates through the stomata, destruction of the cells being first noticeable in the neighborhood of these openings; but it



IG. 1.—The action of sulphuric acid on plant tissues: (3) A, section of leaf of *Brassica arvensis* plant grown in the field during the winter (in England): *a*, hair on upper epidermis; *b*, stoma. B, section of leaf of field-grown plant one hour after it was sprayed with a 3 per cent solution of sulphuric acid: *a*, stoma through which the acid has penetrated and killed some cells. C, leaf similar to B, but showing the effect of the acid three hours after spraying: *a*, stoma; *b*, vascular bundles, diagrammatic. The acid has killed the upper half of the leaf, but the amount of the spray applied was not sufficient to kill the whole leaf. D, section of leaf three hours after it was sprayed with a 5 per cent solution of sulphuric acid: *a*, vascular bundle, diagrammatic. The whole leaf has collapsed as the amount of spray exceeded the quantity which the leaf was able to withstand. Note that the epidermis is the least injured part of the leaf

also seems to penetrate through the epidermal cells, as destruction was soon noticed in cells between the stomata. Figures 1 to 4 show the gradual increase of destruction caused by the penetrating acid.

(2) The sulphuric acid spray decomposes the chlorophyll as it unites with the magnesium atom of the chlorophyll molecule. (Fig. 1, B.) In order to prove this action of the acid, a few drops of 1 per cent solution of sulphuric acid were added to an alcoholic extract of chlorophyll from nettle leaves. It was found that four drops of this acid to five c. c. of chlorophyll extract immediately changed the chlorophyll color from deep green to yellowish green. This change in color indicates that the chlorophyll was split into phaeophytin and magnesium sulphate. According to Willstätter (*40*) this reaction may be indicated by the formula:

$$C_{55}H_{72}O_5N_4Mg + H_2SO_4 = C_{55}H_{72}O_5N_4H_2 + MgSO_4, \text{ or}$$
$$\text{Chlorophyll} + \text{sulphuric acid} = \text{phaeophytin} + \text{magnesium sulphate}$$

Only a very small amount of highly diluted acid is necessary to bring about this reaction, since the amount of chlorophyll in leaves is less than 1 per cent of the dry weight.

(3) It breaks up the chloroplasts. In sections of unsprayed leaves and in cells not yet affected by the acid the chloroplasts were plainly visible. In cells penetrated by the acid the cell contents formed a deeply stained mass in which no chloroplasts could be seen. (Fig. 1, C.) Apparently the acid breaks up the structure of the plastids.

(4) The acid does not destroy the cell walls, at least not those of the epidermis. Sulphuric acid is generally known to be very corrosive. It would seem then that it would "burn" the cell wall as it does clothes and other objects. But Figure 1, A to D, inclusive, shows that the epidermal cells are the least injured parts of the sprayed leaves. This may be explained by the fact that sulphuric acid of low concentration does not dissolve cellulose, which is regarded as the principal constituent of the cell walls. (Fig. 1, D.) The cell walls of the spongy parencyhma of sprayed leaves of pot-grown plants sometimes seemed to be destroyed by the spray. These delicate cell walls are supposed to be built up mostly of pectin (or pectin in combination with calcium) compounds which are dissolved by weak solution of sulphuric acid. However, the destruction of these leaves after the spray was applied was so rapid and complete that the fate of the cell walls was difficult to determine.

Further, it was found that plants grown in the greenhouse were easily destroyed by a 2 per cent solution while plants in the field which had been growing during the winter (in England) required no less than a 5 per cent solution to kill them. The same quantity of spray was used in all cases. As the acid can not evaporate, the plants must be able to absorb a certain amount without being harmed. Death follows spraying only when the sprayed quantity exceeds this amount.

Analyses of the plants showed that the field-grown plants had a far greater amount of dry matter, especially of ash, than the greenhouse-grown plants. This suggested that up to a certain point some of the constituents of the ash were able to neutralize the acid.

It was further found that the leaves of the greenhouse-grown plants changed in anatomical structure with their height above the cotyledons. They became more compact up to the fifth or sixth leaf, the number developed during the time of the investigations.

In reports on spraying experiments it is frequently stated that weeds are most difficult to kill when sprayed in the late rosette stage. If the leaves become more compact in anatomical structure from the cotyledons upward it perhaps would be possible to explain their resistance by changes in anatomical structure accompanied by an increased amount of ash and dry matter. Further work is needed, however, to clear up this point.

Observations clearly indicate that grain crops are unharmed by a spray of sulphuric acid. Their resistance is due to a cutin layer which prevents the sprays from adhering to the plants. The concealed growing point is an additional protection, as several workers have pointed out. In field experiments it is always observed that the ends of the leaves of the grain plants are "burned," the day after spraying has been performed. The lower parts of the leaves are generally unharmed. The writer (*3*) has found this due to the fact that the cutin layer on the lower surface of the leaves is less protective than that on the upper surface. When the leaves of the grain plants have reached a certain size the ends turn over, so that the lower surface comes uppermost. Hence, this part of the leaf is hit by the spray and destroyed. The larger the grain plants are when sprayed the greater is the killed portion of the leaf. In windy weather the sprays will adhere more easily to the lower surface of the leaves and injure the crop.

In some crock cultures (*3*) a 10 per cent solution was sprayed on oats and barley without causing more harm than the weaker solution, as only a very small amount adhered to the plants. It was further found that the amount of spray per unit of area had some influence on the injury. When the commonly used quantities were sprayed only a few leaves were harmed; if larger amounts were used more leaves were injured. The smaller the grain plants and the more vertical their growth the less was the injury.

Peas (*Pisum sativum*), and red clover (*Trifolium pratense*), are found to be uninjured by sprays of sulphuric acid. The writer (*3*) has pointed out that red clover is protected against the sulphuric acid by dense hairs on the leaves. However, the cotyledons are unprotected and therefore are injured. Field trials have shown that a spray of sulphuric acid in a grain field, in which clover seed has been sown, does not harm the clover plants provided they have developed some true leaves. The leaves of peas are rather waxy, so that the spray does not adhere to them.

In some water cultures where seedlings of barley and beans were placed in a full nutrient solution, to which were added increasing amounts of sulphuric acid, the writer (*3*) found a concentration of the acid of 1:20,000 did not injure the plants. Weaker solutions seemed to stimulate the growth. (See fig. 2.)

EXPERIMENTAL WORK

The experiments here reported were conducted to determine the influence of various environmental factors, and of the structure and composition of the plants, upon the effectiveness of sulphuric acid as compared with a solution of iron sulphate when used as weed sprays.

MATERIALS AND METHODS OF PROCEDURE

Plants of field mustard, *Brassica arvensis* (L.) Ktze., and Cornellian oats, *Avena sativa* L., were grown in glazed crocks filled with soil. When the plants had grown to the desired size, they were sprayed

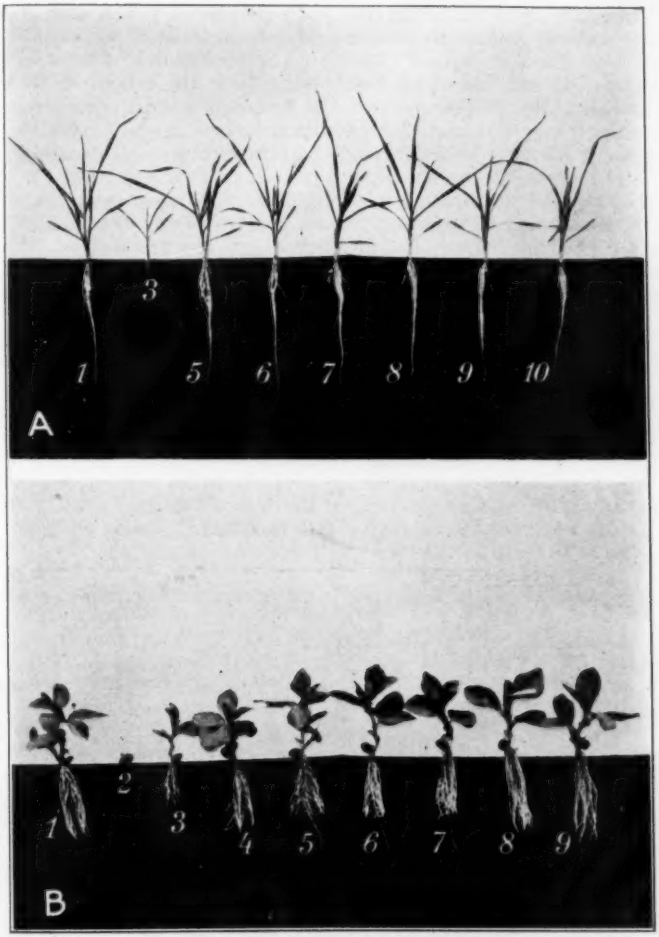


FIG. 2.—Effect of sulphuric acid on plant growth: A, Plants of barley grown in full nutrient solution to which were added increasing amounts of sulphuric acid; B, plants of beans (*Vicia faba*) grown in solutions similar to those in A
Amount of sulphuric acid in solution: 1, 0:1 (no acid); 2, 1:2,500; 3, 1:5,000; 4, 1:10,000; 5, 1:20,000; 6, 1:80,000; 7, 1:320,000; 8, 1:1,128,000; 9, 1:5,120,000; 10, 1:20,480,000
The concentration 1:20,000 had no injurious effect on the plants; weaker solutions seem to have stimulated the growth

with a solution of iron sulphate and dilute sulphuric acid and thereafter placed in glass chambers, where the atmosphere was kept at varying degrees of saturation. Observations were then made on the

effect of the sprays on the plants. The leaves of mustard from some of the crocks were analyzed for dry matter and ash content. Samples of leaves were embedded in paraffin for study of structure.

In order to test the influence of the water content of the soil on the effect of the sprays, plants were grown under three conditions of soil moisture content. For this purpose glazed gallon crocks filled with sifted silt loam, into which had been mixed 10 per cent coarse sand, were used as culture vessels. The crocks held 4 kgm. of soil. The moisture content of the soil, at the time the crocks were filled, was found to be 8.3 per cent in the first experiment, and when the experiment was repeated 12.1 per cent.

In order to secure a uniform distribution of water throughout the soil, a special method was devised for watering the crocks (*5*). The water was applied through a small flower pot placed in the upper soil of the crock. From the flower pot the water was distributed laterally through four radiating "arms" of coarse sand which were inserted about 1 inch below the top of the soil. This method provided a very uniform distribution of soil moisture.

The seeds of mustard and oats were sown February 8, 1926. The plants in each crock were thinned to four oat plants and eight mustard plants. In the repeated experiment the plants were sown on March 22.

In order to obtain an even germination, all of the cultures were at first watered uniformly with a sprinkling can. This method was continued until the cotyledons were well developed. After this the cultures were divided into three lots and the water content was maintained at 15, 30, and 45 per cent of the water-holding capacity of the soil as determined by Hilgard's (*16*) method. Table 4 shows the moisture of the soil of the series in the two experiments.

TABLE 4.—*Moisture content maintained in soil in culture crocks containing mustard and oats*

Test series and experiment Nos.	Average original moisture content of soil	Average weight of water-free soil	Average water-holding capacity of soil	Moisture content of soil maintained during experiments	Quantity of water to be added to soil of original weight of 4 kgm.	Weight of soil plus moisture per crock
Test series No. 1:	*Per cent*	*Kgm.*	*Per cent*	*Per cent*[a]	*Gm.*	*Kgm.*
Experiment 1				15	70	4.070
Experiment 2				15	[b]−10	3.990
Test series No. 2:						
Experiment 1				30	460	4.460
Experiment 2				30	460	4.460
Test series No. 3:						
Experiment 1	8.3	3.668	41.9	45	860	4.860
Experiment 2	12.1	3.516	47.2	45	930	4.930

[a] Indicated as percentage of water-holding capacity.
[b] Crock allowed to evaporate 10 gms. of water in order to come down to 15 per cent moisture.

The water content of the crocks was maintained at a uniform stage by keeping the weight of the crocks constant. As long as the plants were small, so that the water content changed but slowly, the crocks were weighed every second or third day depending on the temperature; but as the plants grew larger it was necessary to water them every day, especially those in medium moist and wet soil.

The average quantities of water lost by the different cultures varied. In experiment No. 1 of series 1, 2, and 3, for the period February 22 to March 14, the losses were 370, 950, and 870 gm., respectively. In experiment No. 2 the temperature was higher, so that the loss of water was greater. During the period April 4 to 22 the loss for experiment No. 2 in series 1, 2, and 3, was 380, 1,260, and 1,170 gm., respectively. These losses represent evaporation from the surface of the soil as well as transpiration by the plants.

As the season gave rather insufficient sunlight for growing strong plants, four 60-watt electric lights were mounted over the cultures and turned on from sunset until midnight.

The temperature of the greenhouse was held at 10° C. during the night. During the daytime the temperature rose depending on the amount of sunlight. Ventilation often proved insufficient to keep the temperature below 16° to 18° C. As a result the plants grew very rapidly and became more succulent than if they had been grown in the open.

SPRAYS USED AND THEIR APPLICATION

The effect of a spray depends to a certain degree on its concentration. If its effect is to be studied the most reliable data may be obtained by the use of a solution of low concentration as differences are most easily observed with such a solution. For this reason, and as the plants were rather succulent, sulphuric acid in concentrations of 1, 1.5, and 2 per cent by weight was used in the present study. The iron-sulphate solutions used in experiment No. 1 were always five times stronger than the sulphuric-acid solutions with which they were to be compared, namely 5, 7.5, and 10 per cent. The writer's experience in field trials indicates that a 20 per cent solution of iron sulphate is about as effective a weed spray as a 4 per cent solution of sulphuric acid. In some of the later applications in experiment No. 2 the concentration of the iron sulphate was increased to 15 per cent.

The plants were sprayed when they had developed four leaves which quite generally is regarded as the best time for this operation. For applying the sprays two types of atomizers were used. Since the sprayers were mounted on graduated cylinders it was easy to determine the amount of spray used. As nearly as possible 1 gm. per square decimeter was applied, equivalent to 1,000 liters per hectare or 107 gallons per acre, which is the amount generally applied under field conditions. The sprayings were performed during the forenoon when the temperature of the greenhouse was about 16° to 18° C. Each culture to be sprayed was divided by a screen of cardboard into two equal parts. One side was sprayed with sulphuric acid and the other with iron sulphate solution.

In order to test the possible influence of the humidity of the air on the effect of the sprays, the sprayed plants were exposed to three different conditions of humidity: (1) to a moist chamber of 90 to 100 per cent relative humidity; (2) to a dry chamber of about 30 per cent relative humidity; (3) to a greenhouse room in which the relative humidity was kept at about 60 per cent. The chambers used in these experiments were the same as those described by Muenscher (*26*). The temperature of the chambers was kept low by shading. The humidity of the greenhouse room was regulated by sprinkling with water and by ventilation.

EFFECT OF SPRAYS ON MUSTARD PLANTS WHEN APPLIED UNDER VARIOUS CONDITIONS

From the experiments made in spraying plants with a solution of iron sulphate the following general deductions may be made:

(1) If the humidity of the atmosphere is low, so that evaporation is rapid, white crystals are soon formed on the surface of the leaves. The epidermis under the crystals may show a natural green color or it may be blackened, depending upon the hardiness of the leaves. The blackening of the leaves increases until they are completely black and dry. The petioles are generally not affected until the leaf is completely destroyed, as the spray seems not to adhere so readily to them. If the spray is strong enough, however, both petioles and stems will be killed.

(2) If the humidity of the air is high enough to prevent or greatly retard evaporation, crystals are not formed. The surface of the leaves becomes black under the drops of the spray, and the plants become flaccid. This continues until the leaves are dead.

Plants sprayed with sulphuric acid of sufficient strength react as follows:

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INFLUENCE OF SOIL MOISTURE ON THE EFFECT OF THE SPRAYS

Soil moisture affected the development of the plants and thus indirectly the effect of the sprays. In the soil with the lowest water content, 15 per cent of water-holding capacity, the plants grew slowly. The color of the plants was a deeper green than those in the other series. The leaves were smaller and the internodes were shorter than on plants grown in soil with higher moisture content, and the plants appeared sturdier and were more hairy. In soil with medium water content 30 per cent of the water-holding capacity, the plants grew largest. The color was light green and the plants appeared rather succulent. Plants grown in wet soil, 45 per cent of water-holding capacity, appeared very similar to those grown in the medium moist series, except that they were somewhat smaller.

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As stated before, the plants were exposed to various conditions of atmospheric humidity after they had been sprayed. The influence of the humidity was rather marked.

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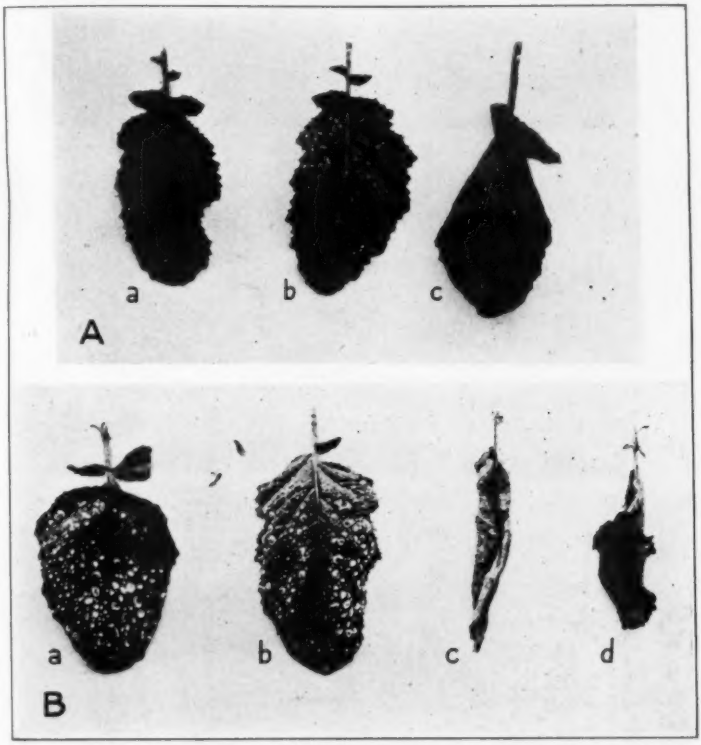

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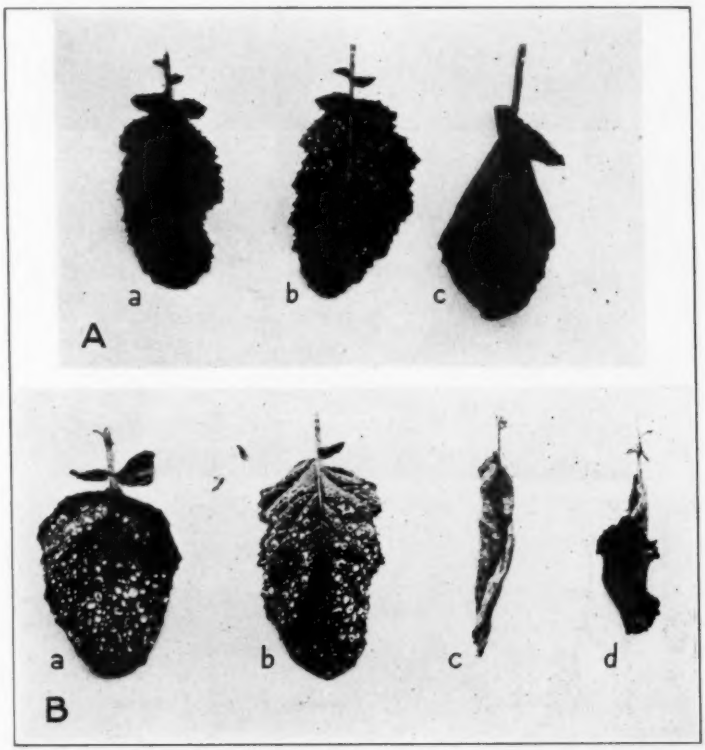

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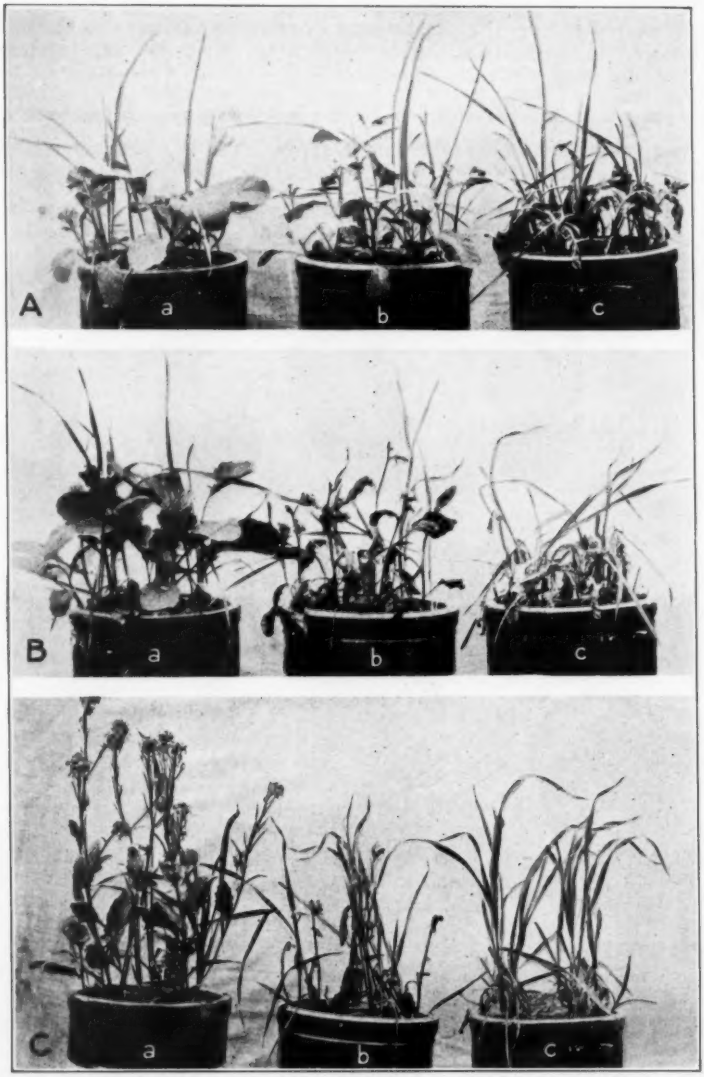

FIG. 4.—Influence of the relative humidity of the air on the effect of sprays. In each case (A, B, and C), *a* is the check crock containing plants which were unsprayed; *b* contains plants which were sprayed with a 15 per cent solution of iron sulphate; and *c* contains plants sprayed with a 2 per cent solution of sulphuric acid: A, appearance of plants one hour after spraying. Plants sprayed with sulphuric acid, crock *c*, are already dead, while the iron-sulphate spray has formed crystals on the leaves of the mustard plants without great damage to them. B, plants one day after spraying. Iron sulphate has affected the plants during the night when the relative humidity was high. C, plants one week after spraying. In the unsprayed pot, *a*, the mustard plants have completely outgrown the oat plants. The iron-sulphate spray has not been able to prevent some of the mustard plants from flowering, while the sulphuric-acid spray has absolutely killed the mustard without harming the oat plants, which are growing vigorously.

Plants grown in dry soil always showed perfectly unharmed leaves. The epidermis was green under the crystals and the leaves were turgid. Plants from medium moist soil appeared to be the most susceptible, as they frequently showed black spots under the crystals. However, the leaves rarely lost their turgor. After the crystals were formed, there seemed to be almost no increase in the injurious effect of the spray on the leaves. The crystals adhered rather loosely to the surface of the leaf. During the following night, when the relative humidity rose to 100 per cent, the action of the spray seemed to continue. By the next morning the effect was very marked, especially on plants grown in medium moist or wet soil. The surface of the leaves under the crystals was a deep black and the discoloration had reached the lower surface of the leaves. Where the crystals were close together, the whole leaf was black and dead. The plants of the dry soil showed greater resistance. Three to four days elapsed after spraying before they were completely destroyed.

In the dry chamber where the humidity was around 30 per cent, the effect of the spray was still less. Plants grown in dry soil were completely unharmed after 24 hours, in spite of numerous crystals covering the leaves. The plants of the moist and wet soil series were more susceptible to the spray and showed some slight injury.

INFLUENCE OF RELATIVE HUMIDITY ON THE EFFECT OF THE SULPHURIC-ACID SPRAY

The action of sulphuric acid was very similar under all conditions of humidity under which it was applied. In the moist atmosphere the action was somewhat delayed, but after two hours exposure to a temperature of 20° C. the leaves were very flaccid and yellow spots appeared on 30 to 50 per cent of the leaf area. Petioles and stems were also affected similarly. On plants sprayed in the greenhouse the effect was visible much sooner, as the water from the dilute spray evaporated, so that the concentration of the acid increased. After a period of five to six hours the leaves were almost completely dried out. In the dry atmosphere the effect of the spray was visible sooner after its application. The leaves of the mustard plants were dead and dry after four hours.

Influence of Temperature on the Effect of Sprays

In the second series of experiments an attempt was made to determine the influence of the temperature on the effect of the sprays. A 2 per cent solution of sulphuric acid and a 15 per cent solution of iron sulphate were used. As the experiments under higher temperature were carried out inside the greenhouse, and those under lower temperature were carried out in the open during cool days, the influence of the temperature was interfered with by the influence of the humidity of the air. However, under field conditions these factors are inseparable.

INFLUENCE OF TEMPERATURE ON THE EFFECT OF IRON SULPHATE SPRAY

At an average temperature of 6° C. and a relative humidity of 78 per cent the action of a solution of iron sulphate had a greater effect than at 30° and a relative humidity of 52 per cent. At the

lower temperature the spray did not evaporate in four hours, so that the crystals when formed had blackened the leaves. At the lower temperature the leaves frequently became flaccid and blackened, which rarely was the case at higher temperature, when the crystals were formed after 15 to 20 minutes.

INFLUENCE OF TEMPERATURE ON THE EFFECT OF SULPHURIC-ACID SPRAY

The action of sulphuric acid was very markedly affected by the temperature. In medium moist soil, the action at the higher temperature, 30° C., was very rapid indeed. After 15 minutes the leaves began to wilt and small yellow spots appeared. In 30 minutes the petioles turned yellow and the stems began to bend over. After one hour the leaves were completely wilted and 50 per cent of the leaf area was yellow or brown. Four hours was time enough to dry the leaves. The plants in the dry soil were affected somewhat more slowly than those in the wet or medium moist soil, but after an hour the difference between the series was hardly detectable. At the lower temperature, 6° C., the action was much delayed. Wilting was not observed until two hours after spraying. Small yellow spots appeared at the same time. After five hours the effect was not quite so marked as after one hour at 30° C. After 24 hours the plants were dead but not dry.

Influence of Rain on the Effect of the Sprays

Rain, falling after spraying is performed, may diminish or inhibit the effect of the operation, as the sprayed solutions will be washed off the plants. However, if the destruction of the plants by the sprays has proceeded beyond recovery when the rain occurs, the influence of the rain is negligible.

Experiments were conducted to test the time necessary for a spray to injure the plants beyond recovery. The plants were sprayed inside of the greenhouse and after a certain time, ranging from 30 minutes to 6 hours, sprinkled with a watering can. The temperature of the greenhouse was around 20° C. and the relative humidity around 55 per cent. It was found that plants sprayed with a 15 per cent solution of iron sulphate were almost unharmed when the spray was washed off three to six hours later. The leaves on plants grown in moist soil were blackened to some extent, but all plants recovered completely. Plants sprayed with a 2 per cent solution of sulphuric acid recovered when sprinkled 30 minutes after they had been sprayed, but if the sprinkling was done one hour after spraying the plants died. However, on the recovering plants, the top buds were destroyed, which means a great check to the plants.

ADDITIONAL TEST OF THE EFFECT OF SOLUTIONS OF IRON SULPHATE AND SULPHURIC ACID ON PLANT TISSUES

In order to obtain additional evidence of the relative rapidity of the action of iron sulphate and sulphuric acid upon plant tissues the following experiments were performed with leaves of water weed, *Elodea canadensis* Rich. The easily observed streaming of the protoplasm in the leaf cells of *Elodea* gives a good indication of unharmed cells. Injury to the cells results in a cessation of the protoplasmic movement.

Leaves of the plant were mounted on cover glasses by using vaseline at one end. Hollow-ground slides such as are used in bacteriological work were used as containers for the solution to be tested, so that the leaf could be observed at any time during the experiment. The time of cessation of streaming was noted, after which the leaf was transferred to a solution of half the strength for half an hour and thereafter into water. After half an hour the leaves were reexamined and the effect noted. The following solutions were used: 10 and 20 per cent solutions of cane sugar, 5 and 10 per cent solutions of iron sulphate, and 0.5, 1, and 1.5 per cent solutions of sulphuric acid. The experiment was conducted at room temperature, about 20° C.

In a 10 per cent solution of cane sugar the streaming continued undisturbed for six hours. No plasmolysis was observed during this time. In a 20 per cent solution plasmolysis was induced and streaming ceased after about 20 minutes, probably owing to the increased viscosity of the cytoplasm. After the cells were transferred to a weaker solution of sugar and then to water they were deplasmolysed and some streaming was noted, indicating that the cells were unharmed. Cells were kept plasmolysed up to four hours with similar results. Plasmolysis did not harm the cells under these conditions.

In a 5 per cent solution of iron sulphate the cells did not become plasmolysed. The streaming began to decrease after about one hour but continued for about two hours. After the cells were placed in water they were tested in a 20 per cent solution of cane sugar. Some cells became plasmolysed after being in the solution of iron sulphate for three hours. The chloroplasts were still green but appeared massed together. A 10 per cent solution of iron sulphate caused plasmolysis after 10 to 15 minutes, but streaming continued up to two hours. The cells were not deplasmolysed when placed in water after they had remained in a 10 per cent solution of iron sulphate for three hours. The chloroplasts were of a natural color and size but clustered together in the plasmolysed cells.

Streaming was never observed when the cells were placed in a 1.5 per cent solution of sulphuric acid. The time necessary to place the slide under the microscope was apparently long enough to stop the movement. After a minute or more the chloroplasts became yellow. If the leaves were then placed in water and thereafter into a 20 per cent solution of cane sugar no plasmolysis was observed. In a 1 per cent solution of sulphuric acid the streaming was observed for 20 to 30 seconds. After two minutes the chloroplasts became yellow and the cells were dead. When the leaves were placed in a 0.5 per cent solution of sulphuric acid, streaming was observed for from two to four minutes. After five to seven minutes the chloroplasts were yellow and the cells showed no sign of plasmolysis when placed in a 20 per cent solution of cane sugar. In no case did a sulphuric-acid solution cause plasmolysis of the cells.

ANALYSES OF PLANTS

In a previous study the writer (*3*) found a correlation between the chemical composition of plants and the strength of sulphuric acid necessary to kill the plants. It was also found that the farther away they were from the cotyledons the more compact was the structure of the leaves of pot-grown mustard plants. In order to

determine whether the difference in the chemical composition of the later formed leaves was sufficient to explain the frequently reported hardiness of the plants in the late rosette stage, against sprays, leaves were analyzed for dry weight and ash content.

The mustard plants from five cultures of each series were harvested for analysis. As each culture had 8 plants, 40 plants of each series were analyzed. The green weight, dry weight, and total ash content were determined for the successive leaves of the plant beginning at the cotyledons. The results of these analyses are recorded in Table 5. This table shows that the plants in the dry soil had the largest amount of dry matter and ash, expressed in per cent of green weight.

TABLE 5.—*Analyses of Brassica arvensis plants in rosette stage, grown in greenhouse from February 8 to March 15, 1926*

[Figures represent total weight of 40 plants]

Leaves analyzed	Green weight (grams)	Dry weight (grams)	Percentage dry weight	Ash (grams)	Ash in percentage of green weight	Ash in percentage of dry weight
Series 1 (plants grown in soil saturated to 15 per cent of water-holding capacity):						
Cotyledons	3.75	0.370	9.87	0.1155	3.08	31.21
First leaf	5.5	.568	10.32	.1575	2.86	27.72
Second leaf	8.7	1.122	12.89	.246	2.83	21.92
Third leaf	6.1	.994	16.30	.166	2.72	16.70
Series 2 (plants grown in soil saturated to 30 per cent of water-holding capacity):						
Cotyledons	8.4	.534	6.36	.185	2.20	34.64
First leaf	12.9	1.022	7.92	.2655	2.06	25.98
Second leaf	23.4	2.017	8.62	.475	2.02	23.55
Third leaf	22.9	2.238	9.77	.4095	1.78	18.28
Fourth leaf	10.0	1.181	11.81	.175	1.75	14.81
Series 3 (plants grown in soil saturated to 45 per cent of water-holding capacity):						
Cotyledons	8.9	.572	6.62	.191	2.14	33.39
First leaf	11.0	.956	8.96	.241	2.19	25.31
Second leaf	17.7	1.615	9.12	.3795	2.14	23.44
Third leaf	21.8	2.287	10.48	.425	1.95	18.58
Fourth leaf	12.0	1.522	12.68	.220	1.83	14.45

TABLE 6.—*Analyses of Brassica arvensis plants in rosette stage, grown in greenhouse from March 22 to April 22, 1926*

[Figures represent total weight of 40 plants]

Leaves analyzed	Green weight (grams)	Dry weight (grams)	Per centage of dry weight	Ash (grams)	Ash in per centage of green weight	Ash in per centage of dry weight
Series 1 (plants grown in soil saturated to 15 per cent of water-holding capacity):						
Cotyledons	3.5	0.335	9.57	0.105	3.00	31.34
First leaf	4.9	.537	10.96	.128	2.61	23.83
Second leaf	7.1	.852	12.00	.172	2.42	20.18
Third leaf	6.0	.806	13.43	.144	2.40	17.87
Fourth leaf	2.7	.382	14.15	.059	2.18	15.45
Series 2 (plants grown in soil saturated to 30 per cent of water-holding capacity):						
Cotyledons	9.0	.536	5.95	.172	1.91	32.08
First leaf	13.8	1.086	7.87	.245	1.78	22.56
Second leaf	21.9	1.755	8.01	.334	1.52	19.03
Third leaf	25.1	2.507	9.98	.425	1.69	19.95
Fourth leaf	16.4	1.945	11.86	.312	1.90	16.04
Series 3 (plants grown in soil saturated to 45 per cent of water-holding capacity):						
Cotyledons	7.8	.524	6.72	.175	2.24	33.39
First leaf	12.8	1.080	8.44	.247	1.93	22.83
Second leaf	19.9	1.780	8.94	.396	1.99	22.25
Third leaf	20.8	2.252	10.82	.391	1.87	17.37
Fourth leaf	13.5	1.630	12.07	.241	1.78	14.78

TABLE 7.—*Amount of dry matter and ash per unit of leaf area of Brassica arvensis plants*

[Figures are based on 40 plants]

Leaves analyzed	Area (sq. dcm.)	Dry matter (grams)	Ash (grams)	Quantity per sq. dcm. of leaf area of—	
				Dry matter (grams)	Ash (grams)
Series 1 (plants grown in soil saturated to 15 per cent of water-holding capacity):					
Cotyledons	0.99	0.335	0.105	0.338	0.016
First leaf	1.42	.537	.128	.378	.090
Second leaf	3.16	.852	.172	.269	.054
Third leaf	3.16	.806	.144	.255	.045
Fourth leaf	1.10	.382	.059	.347	.054
Total or average	9.83	2.912	.608	.296	.062
Series 2 (plants grown in soil saturated to 30 per cent of water-holding capacity):					
Cotyledons	1.98	.536	.172	.271	.087
First leaf	3.67	1.086	.245	.296	.067
Second leaf	7.95	1.755	.334	.221	.042
Third leaf	10.85	2.507	.425	.231	.039
Fourth leaf	8.28	1.945	.312	.234	.037
Total or average	32.73	7.829	1.488	.239	.045
Series 3 (plants grown in soil saturated to 45 per cent of water-holding capacity):					
Cotyledons	1.82	.524	.175	.288	.096
First leaf	4.00	1.080	.247	.270	.061
Second leaf	6.70	1.780	.396	.265	.059
Third leaf	7.96	2.252	.391	.283	.049
Fourth leaf	6.52	1.630	.241	.250	.037
Total or average	27.00	7.266	1.450	.269	.054

The plants in the medium moist soil showed the lowest content of dry matter and those in the wet soil an intermediate amount. However, the difference between plants of medium moist and wet soil was not very marked. Expressed in per centage of green weight, the dry matter of the leaves increased from the cotyledons upward while the ash content decreased.

Analyses of plants grown in the second experiment checked very closely with those of the first experiment. (See Table 6.)

The analyses of the plants in the first experiment gave results which show a marked difference in the chemical composition of the leaves, depending on their position above the cotyledons. But figures expressing percentages may give a false impression. If the action of sulphuric acid is diminished or inhibited by its absorption into the plant tissue (this action depends upon the amount of dry matter and ash of the leaves), it is obvious that it is the amount of dry matter and ash per unit of area which is important. The spray is always applied in a given quantity per unit area. Thus it was necessary to measure the leaf area in order to calculate the amount of dry matter and ash per unit of area. In the second experiment the leaf areas were determined by measuring tracings of the leaves with a planimeter. The results are recorded in Table 7.

Table 7 shows that there was a difference between the plants of the three series. Plants grown in the dry soil had the highest average amount of dry matter and ash per square decimeter of leaf area. Plants grown in medium moist soil had the lowest and those in

wet soil had an intermediate amount of dry matter and ash. Thus far the figures agree with those expressing the amount in percentage of green weight. However, instead of increasing from the cotyledons upward, the dry matter per square decimeter actually decreases. The ash content also decreases, even to a more marked degree. The results of the analyses recorded in Table 7 seem to indicate that the resistance of the mustard plants, in late rosette stage, can not be due to the increased amount of dry matter and ash in the upper leaves of the plants.

DISCUSSION

THE EFFECT OF THE SPRAYS

The experiments show that there is a very marked difference in the action of the sprays used. The cells of a plant are able to endure a relatively high concentration of iron sulphate for a considerable time, while a very dilute solution of sulphuric acid kills the cells almost instantly. On the other hand, iron sulphate seems to be able to act for some distance. For instance, if plants are sprayed, the spray is seen to adhere to the leaves, but rarely to the petioles or the stems. Nevertheless, after some time, especially on plants placed in the moist chamber, it was found that both petioles and stems were black and killed. The salt seems to move through the tissues of the leaves. With sulphuric acid this was never observed, at least not with the quantities used as sprays, the acid acting solely at the place where it hits the leaf. On the other hand, it seems to adhere more readily to petioles and stems, as these were observed to be killed as rapidly as the leaves. It is shown, however, that sulphuric acid under certain circumstances may move through the leaves. If the tip of a leaf is placed in a solution of the acid, the whole plant will be killed. In the quantities applied as sprays the acid seems to be absorbed by the tissues without being transported to other parts of the plants.

The action of iron sulphate upon plant tissues is not clearly understood. Several theories have been advanced. Olive (*27*) believes that "death is due to osmotic properties rather than to absorption of the chemical into the leaves." The water, according to his theory, is drawn out of the leaves by the flakes of dried salt on the surface. He believes drying of the solution to be a necessary process. On the contrary, Schultz (*34*), citing some investigations by Stender (*35*), states that leaves are unharmed after being plasmolysed for several hours by various salt solutions, if the salt is then washed off. Earlier experiments by the writer (*3*) in which plant tissues were treated with strong solutions of sodium chloride, confirm these results. Schultz finds that iron sulphate has a certain harmful effect, but declares that he does not know how it acts.

The blackening of the leaves of sprayed plants is explained by Olive (*27*) as being due to the formation of sulphides in the leaf or "union of * * * sulphate with the living substance." Plants of the mustard family are characterized by the presence of mustard oils which contain a relatively high per cent of sulphur. This fact is probably the basis for Olive's interpretation of the action of iron sulphate. Schultz (*34*), however, denies that this reaction takes place. He believes that the blackening of the leaves is due to a reaction between iron and tannic acid of the tissues. Whatever the action

may be, one thing is clear, and that is that the action is a slow one. Leaves of *Elodea* endured a 10 per cent solution for two hours.

The experiments show that a solution of iron sulphate may easily kill the tissues without causing plasmolysis. A 5 per cent solution completely destroyed the mustard plants in 24 hours in the moist chamber. But as such a solution did not cause plasmolysis in leaves of *Elodea*, even though most of the cells were killed within three hours, probably it acted in a similar way on leaves of mustard. In the moist chamber no evaporation took place, so that an increase in concentration was out of the question. The experiments thus support the view of Schultz that it is the chemical action of the spray that kills the plants.

The action of sulphuric acid is easier to interpret. It is the high hydrogen-ion concentration which is injurious. Brenner (*10*, *11*) has studied extensively the effect of various acids on plant cells. He found them injurious in proportion to their acidity, i. e., in proportion to the actual hydrogen-ion concentration of the solution. He placed pieces of epidermis from several plants in acids of various strengths and tested their effect after a certain time. For instance, he found that a $\frac{M}{50}$ solution of sulphuric acid, equivalent roughly to a solution of 0.2 per cent killed cells of *Brassica oleracea* in two minutes. A $\frac{M}{1300}$ solution killed the cells in four hours. The hydrogen-ion concentration of this solution he gives as 1.4×10^{-3}. Expressed in a more common term of hydrogen-ion concentration, the acidity was approximately pH 2.85. Calculated in percentage this solution contained about 0.0075 per cent sulphuric acid. These figures, as well as the experiments with *Elodea* reported in this paper, show clearly that the protoplasm is very susceptible to an acid solution. However, it is not possible to destroy weed plants with a sulphuric-acid solution of 0.2 per cent strength, in spite of the fact that Brenner has shown that it will kill cells in two minutes. In these experiments a 2 per cent solution was necessary; in field trials in spring-sown grain crops a 3.5 to 4 per cent solution was required. Rabaté (*31*) found that a 10 per cent solution was just strong enough to kill weeds in winter wheat sprayed in early spring. This makes it clear that plants are able to absorb some acid and neutralize it, so that they will be killed only when the absorbing capacity has been exceeded. Only a small amount can adhere to the plants. The necessity of increased strength of spray when winter-grown weeds are to be eradicated indicates that the difference in resistance is to be looked for in the difference in anatomical structure of plants grown under various conditions. The illustrations in Figure 5 are cross sections of leaves of *Brassica arvensis* grown under various conditions. Plants grown under the most severe conditions have the most compact leaf structure. It is the cell walls which are affected. This makes it clear that the protecting capacity is situated in the cell walls, which seem to be able to absorb and neutralize at least some of the acid.

The great resistance of plants to sprays frequently observed in the late rosette stage, seems not, as far as these experiments show, to be due to the increased amount of dry matter and ash in the upper leaves, as earlier experiments suggested. There is actually a decrease

in dry matter and ash content per unit of leaf area from the lower leaves upward. However, this increased resistance is reported, especially when sprays of salt solutions of various kinds have been used. Resistance to such sprays is a question to a large extent of the thickness and modification of the outer cell walls of the epidermis, which are greatly influenced by periods of drouth such as very commonly occur in spring and early summer. Still another explanation may be found. As long as the plants are small, the leaves are growing more or less horizontally. A spray at this time will adhere to the plants rather easily and in large amounts. When the plants grow larger and are in competition with grain plants the upper leaves grow more nearly vertically. A spray applied under these conditions adheres with more difficulty than on horizontal leaves and the amount of spray adhering per unit of leaf area will be less. The larger the leaves

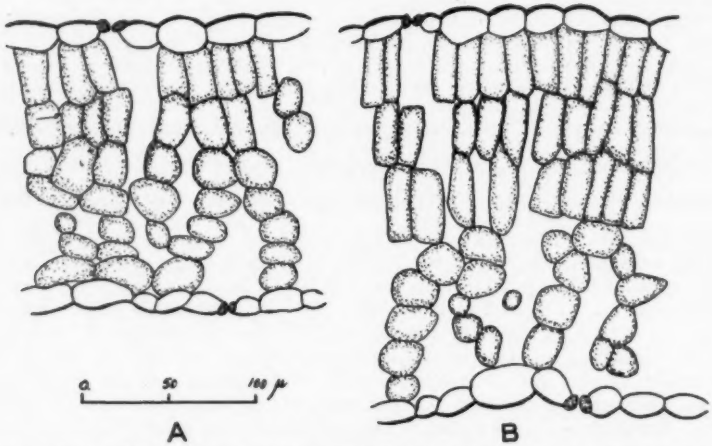

FIG. 5.—Cross section of leaves of *Brassica arvensis:* A, leaf of plant grown in medium moist soil, showing rather loose structure, weakly developed palisade tissue, and large air spaces; B, leaf of plant grown in dry soil, the structure of which is more compact, which accounts for the greater resistance of the plants to sprays

Both drawings were made with the aid of a camera lucida and are of the same magnification

of crowded plants, the more will these plants protect each other. Thus early spraying is always more destructive to the weeds.

INFLUENCE OF WEATHER ON THE EFFECT OF SPRAYS

The experiments confirm the reports of field trials of weed eradication, namely, that rapidly growing plants are most susceptible to sprays. The most suitable weather conditions for spraying, according to general opinion, obtain on a dry, sunshiny, calm day. It is significant that Bolley (*6*) is the only one who recommends humid weather rather than dry weather for the application of weed sprays. Working in North Dakota he found the wettest and most rapidly growing condition to be the most satisfactory, and states that "it is useless to expect desirable results by spraying in droughty, windy weather." He used several salt solutions as sprays.

Experiments described in the present work fully confirm the statement of Bolley. The action of a solution of iron sulphate under dry

conditions is very weak, especially on plants grown in dry soil. The salt crystallizes before the leaves are injured by the spray. In a moist atmosphere the spray acts rather efficiently. It is clear then that iron sulphate when sprayed under dry conditions begins to act when the relative humidity has increased to a certain point, which probably is near 100 per cent or at the dew point. For that reason good results may generally be expected in humid regions, and in dry regions only if the relative humidity happens to be high. With this point clear, it is of interest to examine the relative humidity in the agricultural regions of the United States during May and June, the time when weed sprays are usually applied. Day (*12*) in an extensive report on the relative humidity of the United States, shows very clearly that with the exception of the coastal regions, the relative humidity is very low during the spring and early summer.

Ward (*38*) in a paper discussing the relative humidity of the United States cites Hann (*15*), who states that the relative humidity of the New England States, one of the humid regions of the United States, is lower than it is in western Europe. He tries to explain this condition as an effect of prevailing winds. This low relative humidity explains why the use of iron sulphate and other salt sprays against weeds have given much better results in Europe than in the United States.

The action of sulphuric-acid spray upon plant tissues is favored by warm and dry weather. The warmer and dryer the weather the better are the results obtained.

The protoplasm is killed instantly when it comes in contact with sulphuric acid of the strengths used in sprays. The time elapsing from the moment the spray adheres to the plant until the tissues are killed depends upon the rate at which the acid penetrates the tissues. The rate of diffusion increases rather rapidly with rise in temperature. The fact that the rate of action of the spray increased about five times when the temperature was raised from 6° to 30° C. may be accounted for chiefly by the increased rate of diffusion. The action of the acid on the tissues is a chemical one. Rise in temperature increases the velocity of a chemical reaction still more than that of a physical one. Thus an increase in temperature accelerates the chemical action of the spray still more than its diffusion into the tissues. At the higher temperature employed in these experiments evaporation is very much higher than at the lower one. Evaporation of water from the spray increases the concentration of the sulphuric acid and thus increases its action. Another effect of the increased evaporation at the higher temperature is that the sprayed parts of the plant begin to dry up as soon as the protoplasm is killed, or as soon as the plant becomes flaccid. This drying rapidly increases the visible effect of the spray. Taking into consideration these effects of an increase in temperature on the action of the sulphuric-acid spray, its rapidly increasing effectiveness with rise in temperature is readily accounted for.

SUMMARY

Plants of field mustard, *Brassica arvensis* (L.) Ktze, and Cornellian oats, *Avena sativa* L. were grown in pot cultures in the greenhouse.

The soil moisture of the cultures was kept at 15, 30, and 45 per cent of the moisture-holding capacity of the soil. A watering

system giving an even distribution of moisture in the soil was adopted. Mustard plants grown in the dry soil were found to have a much more compact anatomical structure than those grown in medium moist or wet soil.

The plants were sprayed with solutions of iron sulphate varying in strength from 5 to 15 per cent and with solutions of suphuric acid, varying in strength from 1 to 2 per cent. The sprayed amount was 1 gm. per square decimeter (1,000 liters per hectare, or 107 gallons per acre).

The sprayed plants were exposed to three conditions of atmospheric humidity, namely, about 30, 60, and 100 per cent of relative humidity. A solution of iron sulphate was found to be most destructive in an atmosphere containing about 100 per cent relative humidity. Under such conditions a 5 per cent solution completely killed the mustard plants in 24 hours. In dry air, with a relative humidity from 30 to 60 per cent, the solution of iron sulphate sprayed upon the plants evaporated rapidly and salt crystals were formed on the surface of the leaves without injury to the plants. When the relative humidity was allowed to increase to about 100 per cent, the plants were soon killed. This action was easily followed on plants grown in dry soil, while plants grown in moist soil were sometimes injured before the crystals were formed. Solutions up to 15 per cent strength were used without any different effect. As the relative humidity in the United States generally is very low during May and June a spray of iron sulphate will have but a slight effect on hardy plants.

When a solution of sulphuric acid was sprayed on the plants, the mustard plants were killed under all conditions of humidity, but best results were obtained in dry air. Plants grown in moist soil were killed off by a 1.5 per cent solution, while plants grown in dry soil required a 2 per cent solution to destroy them completely. This results indicates that the latter plants were able to absorb some acid without being injured permanently. As a spray of sulphuric acid gives the best results in dry air it is a spray to be recommended for dry regions.

Temperature had a marked influence upon the effect of the sprays. At 30° C. a 2 per cent solution of sulphuric acid killed the plants in one hour, while at 6° the same effect was obtained only after five hours. A 15 per cent solution of iron sulphate was more effective when sprayed at a lower temperature, as the evaporation of the water and crystallization of the sulphate was very slow giving the solution a longer time to act. In no case did a solution of iron sulphate kill the plants in less than 24 hours.

Artificial rain, produced by sprinkling, applied to the plants one hour after they had been sprayed with a 2 per cent solution of sulphuric acid failed to decrease the effect of the spray. Plants grown in moist soil sprayed with a 15 per cent solution of iron sulphate were but slightly harmed when "rain" was applied six hours after spraying. Plants grown in dry soil were unharmed.

In an additional test of the relative rapidity with which solutions of iron sulphate and sulphuric acid act upon living plant cells it was found that protoplasmic streaming in the leaves of *Elodea canadensis* Rich. continued for two hours in a 10 per cent solution of iron sulphate but ceased in 30 seconds in a 1 per cent solution of sulphuric

acid. The cells were killed after having been kept in the iron sulphate solution for three hours or in the sulphuric acid for two minutes.

Analyses of plants indicate that the great resistance to sprays exhibited by plants in the late rosette stage, can not be explained by the increased amount of ash and dry matter in the upper leaves. Other explanations are suggested.

The oat plants were not harmed by the sprays.

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